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THE RELATIONSHIP BETWEEN DIETARY CHOLESTEROL AND BLOOD CHOLESTEROL AND HUMAN HEALTH AND NUTRITION

A Report to the Congress
Pursuant to the Food Security Act of 1985
P.L. 99-198, Subtitle B, Section 1453

DEPARTMENT OF HEALTH AND HUMAN SERVICES
DEPARTMENT OF AGRICULTURE
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EXECUTIVE SUMMARY

P.L. 99-198 calls for an assessment of the existing scientific literature and research on the relationship between dietary cholesterol and blood cholesterol and human health and nutrition, as well as recommendations for studies that may be considered appropriate.

The necessary assessment and planning were conducted jointly by the Department of Health and Human Services and the Department of Agriculture. A comprehensive update of the relevant scientific literature was commissioned (Appendix A); a workshop on dietary cholesterol was convened and a summary of the proceedings and recommendations from that workshop are included (Appendix B); the relevant federal research support was identified and reviewed (Appendix C); and the accompanying report has been prepared.

Elevated blood cholesterol is clearly linked to the risk and causation of atherosclerosis and coronary heart disease; it is not the only risk factor, but it is one of the major risk factors. The blood cholesterol level in people is affected by diet and by such other factors as body weight and genetics. Cholesterol in the diet, but even more the amount and types of fat in the diet, affect the blood cholesterol. In practical terms, the problem is complicated because foods that contain cholesterol also contain fats.

Dietary cholesterol raises blood cholesterol in most people; in many, there is a small or modest response, but in perhaps 10 or 20 percent of adults the increase is more pronounced. Within the population, the prevalence of different degrees of responses is not accurately known. Unsettled questions include the factors affecting the response of an

individual and the reproducibility of an individual's response pattern. In addition to its effect upon the blood cholesterol levels, dietary cholesterol may also play a role in atherogenesis and heart disease through its effects on cholesterol metabolism within the body.

There has been and continues to be considerable federally supported research on dietary cholesterol, blood cholesterol, and human health. Advances in knowledge provoke new questions; the availability of new research techniques allows long-standing questions to be addressed. Research needs continue and research along certain lines must now receive special emphasis:

- The bases for the variability of response in fasting blood cholesterol and lipoprotein levels to dietary cholesterol and the prevalence of different responses.
- Cholesterol metabolism and the influence of dietary cholesterol thereon.
- The effects of dietary cholesterol on the composition and concentration of post-prandial lipoproteins, the variability in their responses, and their interactions with other lipoproteins.
- Interactions of post-prandial lipoproteins and lipoprotein remnants with cells of the arterial wall.

In addition to these investigations in humans, unresolved questions relating to dietary cholesterol, blood cholesterol and atherosclerosis should continue to be addressed in experimental animals.

No single protocol will provide answers to all the questions posed. A broad program of research on dietary cholesterol, blood cholesterol, human health and nutrition will continue. It will emphasize the priority areas identified, without excluding other relevant topics.

INTRODUCTION

This report responds to the directive in P.L. 99-198 that calls for the Secretary of Agriculture and the Secretary of Health and Human Services to "jointly conduct an assessment of the existing scientific literature and research relating to the relationship between dietary cholesterol and blood cholesterol and human health and nutrition" and to recommend further studies that may be considered appropriate.

The Department of Health and Human Services, primarily through the National Heart, Lung, and Blood Institute, and the Department of Agriculture, primarily through the Agriculture Research Service, collaborated in the preparation of this report.

A comprehensive review of the relevant literature by Henry C. McGill, Jr., M.D. was commissioned to update his previous review published in 1979; these reviews are included as Appendices A1 and A2. It is anticipated that the current review will also be published in a widely read scientific journal.

A workshop was jointly convened July 1-3, 1986 at which 24 speakers and more than 100 other experts broadly representative of the involved scientific disciplines addressed the relationship of dietary cholesterol, plasma lipoproteins and atherogenesis. A summary of the extensive discussions and the recommendations derived from that workshop are included as Appendix B. It is anticipated that this report will also be published in a widely read scientific journal.

The federal support for research on dietary cholesterol in Fiscal Years 1984 and 1985 was reviewed. More than 90 projects were identified. They are presented in Appendix C, grouped under topical headings with brief scientific abstracts.

CHOLESTEROL

Cholesterol is a lipid substance found in all animal tissues but never in plants. It is of particular clinical interest because of its relationship to the leading cause of death in America--coronary heart disease. Cholesterol has many roles in normal body function. It is a precursor for adrenal hormones, sex hormones and bile acids, and it is an essential structural component of all cell membranes. Without cholesterol, cells cannot grow or thrive.

Body cholesterol is both derived from the diet and manufactured within the body (endogenous synthesis). Animal foods--beef, pork, poultry, seafood, eggs and milk products--are the main sources of dietary cholesterol. Foods especially high in cholesterol include egg yolks and organ or glandular meats such as liver, kidney, sweetbreads and brain. The average United States diet is estimated to contain 300-600 mg of cholesterol per day. An additional amount, about 700 mg per day, is manufactured within the body, primarily by the liver.

BLOOD CHOLESTEROL AND HUMAN HEALTH

Atherosclerosis, a fatty infiltration of arteries that leads to their narrowing and hardening, underlies the leading causes of death in the Western World. Coronary heart disease (with such manifestations as

angina pectoris, heart attack and sudden death), stroke, and peripheral vascular disease are its main clinical presentations.

It has been repeatedly demonstrated that the probability of developing a heart attack can be predicted by the concentration of cholesterol in the blood of apparently healthy individuals. Elevated blood cholesterol is recognized as a major risk and causal factor in the development of coronary heart disease. The other major risk factors are elevated blood pressure and cigarette smoking, and much evidence indicates that they are substantial risk factors even at blood cholesterol levels below 182 mg/dl. Blood cholesterol for the adult American population averages about 210 mg/dl. Recently it has also been shown that a reduction in blood cholesterol levels leads to a reduced incidence of heart attack and other manifestations of coronary heart disease. It seems that each 1 percent reduction of blood cholesterol reduces the risk for heart attack by approximately 2 percent. Thus, blood cholesterol levels are of paramount importance in the risk of coronary heart disease.

The cholesterol in the blood is carried in several types of lipoprotein particles that are packaged as soluble fat-protein complexes that differ in size, composition and other physico-chemical properties. There is considerable variation, but typically 60-70 percent of cholesterol may be found in low density lipoprotein (LDL), a further 20 percent or so in high density lipoprotein (HDL), and the balance in very low density lipoprotein (VLDL). These fractions have different relationships to coronary heart disease risk. The higher the levels of LDL, the greater the risk. In contrast, HDL levels are inversely related to risk, so that

higher levels are associated with less coronary heart disease. The precise relationship of VLDL to coronary risk remains to be defined.

Although the primary interest is in the cholesterol-heart disease relationship, epidemiological studies have revealed that men with the lowest levels of blood cholesterol have an increased likelihood of manifesting bowel cancer. There is substantial evidence that this is primarily the result of unrecognized, preclinical cancer that has lowered the blood cholesterol, but there is also plausible evidence for a possible secondary factor, that naturally low blood cholesterol levels may be associated with a greater susceptibility to bowel cancer in males. High dietary cholesterol, however, seems associated with increased risk of colon and certain other cancers.

DIETARY CHOLESTEROL, BLOOD CHOLESTEROL AND HEART DISEASE

The relationship between elevated blood cholesterol and the development of atherosclerosis and coronary heart disease is firm. However, the relationship of dietary cholesterol to the foregoing is complicated by several factors. Dietary cholesterol is generally found with dietary fats, which also have effects upon blood cholesterol. Also, dietary cholesterol may play a role in atherogenesis and heart disease through its effects on cholesterol metabolism in the body, independently of its effects upon the blood cholesterol level.

Animal studies: The historical linkage of dietary cholesterol to atherosclerosis dates back to the early part of this century when, shortly after the discovery that the human atherosclerotic aorta contained twenty times as much cholesterol as did the normal aorta, the development of aortic

atherosclerosis was demonstrated in rabbits fed milk, meat and eggs. The effects of these foods were traced to cholesterol by feeding rabbits the pure chemical dissolved in vegetable oil and reproducing identical lesions. Since then, the effects of dietary cholesterol have been duplicated in many other animal species. There is a wide spectrum of sensitivity to dietary cholesterol among animal species; the best approximation to that of man exists in some subhuman primates, but that is no more than an approximation. A signifcant problem has been and continues to be the extrapolation of results in animals to humans.

Studies in humans: The observation that cardiovascular mortality declined in areas of Western Europe during periods of war, when foods containing cholesterol and fat were especially scarce, raised the possibility that these foods were indeed implicated in human atherosclerosis. Several studies have also shown positive correlations between populations ingesting high quantities of cholesterol-containing foods and their incidence of coronary heart disease, but there also have been a few notable exceptions to this observation. Of direct relevance to the U.S. population are the observations in the past two decades of a 40 percent fall in the death rate from coronary heart disease. This has been accompanied by decreases in blood cholesterol levels and reductions in saturated fat and cholesterol intake as well as other life style changes, such as decreased smoking, better management of high blood pressure, and improved treatment of heart disease. Despite these indications, it has been difficult to define the precise quantitative relationship between dietary cholesterol and blood cholesterol levels, particularly in free-living individuals.

Most of the evidence that dietary cholesterol plays an important role in elevating blood cholesterol comes from settings in which the diet can be controlled effectively--from studies in metabolic wards or studies of people living in institutions. In free-living individuals with varied and complex sources of food, the relationship has been particularly difficult to establish. There are a number of reasons for this. Epidemiologic studies are confounded by the effects of other dietary variables on blood cholesterol that make it difficult to isolate the effect of dietary cholesterol. Saturated fat is particularly implicated in this respect, but such other factors as the total fat content of the diet, fatty acid composition and fiber are of importance. In addition, obesity, lack of exercise, and increasing age are associated with increased blood cholesterol levels.

Dietary cholesterol appears to raise blood cholesterol in most people, but there is marked variability in individual responses. Those in whom the increment in blood cholesterol is modest are termed "hypo-" or "low-responders," but 10-20 percent of adults appear to be "hyper-" or "high-responders" and will manifest a more pronounced increase in blood cholesterol. Dietary cholesterol in the latter, if not restricted, could significantly raise blood cholesterol levels and confer increased risk of atherosclerotic disease. Unfortunately, reliable measurement of an individual's response is quite difficult. Some researchers have found the response to be both consistent and reproducible within the individuals, while others have had opposite findings. No simple discrete marker for sensitivity is available, but the response is probably under genetic control. Many diverse genetic and environmental factors thus influence blood cholesterol levels.

The average cholesterol level in middle-aged adult populations at low risk for coronary heart disease is around 160 mg/dl; in a high-risk culture such as the United States, the average level is about 210 mg/dl. It has been calculated that dietary cholesterol contributes about one-fifth or 10 mg/dl to this 50 mg/dl difference between the low- and high-risk populations, the rest being attributed to the other factors identified earlier.

Effects of dietary cholesterol other than on blood cholesterol level: Despite the fact that most observational and experimental investigations of the relationship of dietary cholesterol to atherogenesis have assumed that the effect was mediated by blood cholesterol concentration, dietary cholesterol could affect atherogenesis without altering blood cholesterol levels. Three recent longitudinal epidemiologic studies found significant associations of dietary cholesterol intake with subsequent coronary heart disease independent of blood cholesterol levels, giving credence to this possibility. However, other studies have failed to show an independent association. Dietary cholesterol could influence atherosclerosis irrespective of blood cholesterol levels through its effect on cholesterol metabolism in the body.

CHOLESTEROL METABOLISM

Dietary (exogenous) cholesterol: After its ingestion, dietary cholesterol is solubilized in the intestine and then absorbed. The absorption is influenced not only by the quantity of cholesterol but also by its physical state. The amount absorbed is proportional to the amount eaten, but it is generally agreed that at higher intakes, e.g., greater than 500 mg/day,

further increments have lesser or little effect on the blood cholesterol concentration. When cholesterol is administered in crystalline form, as in some laboratory experiments, its absorption is less than when it is administered in solubilized form as in egg yolks or dissolved in oils; this is one reason that earlier studies of administering cholesterol failed to show a convincing effect.

Absorbed cholesterol circulates in the blood exclusively in particles called chylomicrons. These large lipoproteins enter the blood stream only after meals (post-prandially) and they are absent in blood sampled several hours later, i.e., during a period of fasting. Their fat (but not their cholesterol) is removed on entering blood capillary beds. The residual particle, called a chylomicron remnant, contains all newly-absorbed dietary cholesterol; it is released into the blood and is removed when it passes through the liver. There is increasing evidence suggesting that these remnant particles can lead to atherosclerosis, but much remains to be understood about their role in atherogenesis. They may directly deliver the dietary cholesterol to the cells that constitute the inner lining of the artery. Some recent research shows that remnant lipoproteins are selectively taken up by macrophages, the scavenger cells of the body, which then laden with fat, become transformed into the foam cells that are prominent in atherosclerotic lesions.

Endogenous cholesterol: The liver, the major site for manufacturing cholesterol in the body, is under some degree of regulation from dietary cholesterol. Certainly in animals, a high cholesterol diet will depress liver cholesterol manufacture; a similar inhibitory effect has been

reported in humans, but this does not entirely compensate for the increased cholesterol intake. Cholesterol in the liver can be converted to bile acids, secreted into bile as cholesterol or secreted into plasma within lipoproteins. Bile acids are almost completely reabsorbed by the small intestine, but only 30-60 percent of the biliary cholesterol is reabsorbed, the rest being excreted through the large intestine in a modified form called neutral steroids. Dietary cholesterol probably influences all these metabolic parameters. When excess cholesterol is fed to dogs or rats, the increment is transformed rapidly into bile acids; in humans most excess dietary cholesterol is re-excreted into bile as cholesterol, thus causing supersaturation of bile, which potentially promotes gallstone formation.

Cholesterol is secreted into the bloodstream from the liver within VLDL. The VLDL is then converted in the plasma into LDL, which become the main transporter of cholesterol in the circulation and the main supplier of cholesterol to cells. The role of cholesterol in cellular structure, integrity and growth has been well defined, and the recent Nobel prize-winning work of Brown and Goldstein has described how LDL binds to a receptor at the cell membrane, is carried into the cell and then is broken down, releasing its contained cholesterol. This cholesterol is either incorporated into cell membranes or stored. To prevent excessive increases in cellular concentrations of cholesterol, the cholesterol in the cell reduces the activity of the receptor, thus limiting the amount of further LDL bound and taken up by the cell.

Any excess of cholesterol must be removed from the cell. A mechanism of some kind is required to accept cholesterol secreted by body cells into

the blood stream and a means must be provided for return of cholesterol to the liver for its final excretion from the body. Much research is being directed at the possible mechanisms for this "reversed cholesterol transport." High density lipoprotein (HDL) is generally considered as the possible cholesterol transporter in this regard.

CONCLUSIONS AND RECOMMENDATIONS

Review of the scientific evidence regarding the influence of dietary cholesterol on blood cholesterol, blood lipoproteins, atherosclerosis and coronary heart disease indicates that much is known and there is considerable ongoing research. However, more remains to be learned and must be investigated about the immediate and long-term effects of dietary cholesterol.

The variability of responses to dietary cholesterol in different people--the minimal, moderate and high responders--is known, but the methods for defining the response of a person are complex. The prevalence of different degrees of responders in the population is not adequately characterized. There are reports of both reproducibility and nonreproducibility in the type of response of individuals. The roles of other dietary constituents, genetic predispositions and possible other factors affecting this response are inadequately understood. Therefore,

(1) the bases for the variability of response in fasting blood cholesterol and lipoprotein levels to dietary cholesterol needs to be better understood and the prevalence of different responses needs to be better characterized.

The metabolism of cholesterol in humans is complex. It includes such processes as absorption of dietary cholesterol, synthesis of cholesterol within the body, conversion of cholesterol to bile acids, biliary secretion of cholesterol, actions and regulation of receptors for low-density lipoprotein, accumulation of cholesterol in tissues, and "reverse transport" of cholesterol. Changes in dietary cholesterol may produce long-term changes in cholesterol metabolism and in cholesterol body pools that are not apparent in short-term studies. The complexities of cholesterol metabolism and its control may also be important in the heterogeneity of responses to dietary cholesterol. Therefore,

(2) cholesterol metabolism and the influence of dietary cholesterol thereon are important topics of research.

Man spends much of his life in the non-fasting state, and almost certainly post-prandial lipoproteins play no less a role in atherogenesis than those in the fasting state. The concern that dietary cholesterol may be atherogenic independently of its effect on fasting cholesterol and lipoprotein levels, represents a reflection of the growing role attributed to the post-prandial lipoproteins in atherogenesis. These lipoproteins comprise the chylomicron remnant particles and other particles similar to VLDL in size and density which contain all the dietary cholesterol that is absorbed. They circulate after the ingestion of food and are more commonly found in the bloodstream than those lipoproteins associated with the fasting state. For many years they have been considered atherogenic, but adequate measurements were not made for lack of knowledge and proper methodology. Current advances in the area of molecular biology have allowed the development of analytical tools

that should meet the previous shortcomings. Knowledge of the lipoprotein system has also expanded considerably and provides a more meaningful base upon which to acquire further insights. Therefore,

(3) the effects of dietary cholesterol on the composition and concentration of post-prandial lipoproteins, the variability in their responses, and their interaction with other lipoproteins can now be studied more effectively and are important topics for further research.

Cells lining the arterial walls are involved in the atherosclerotic process. Cholesterol-containing lipoproteins and lipoprotein remnants may have several types of actions on these cells including cytotoxicity, interaction with different types of receptors, formation of foam cells, direct transfer of cholesterol from lipoproteins to cells, and interference with the release of cholesterol from cells. These processes may be intimately involved in atherogenesis and need to be better understood. Therefore,

(4) interactions of post-prandial lipoproteins and lipoprotein remnants with the cells of the arterial wall are an important topic for research.

Many of the unresolved questions relating to dietary cholesterol, cholesterol metabolism and atherosclerosis cannot be studied in humans in a practical manner; they will continue to be addressed in animal models. Although the applicability of such studies to man may remain a problem, some non-human primate models are more similar to humans in their responses to cholesterol feeding and these animals hold the greatest promise. The effects of dietary cholesterol warrant continued study in animal models, as well as in humans.

The relationships of dietary cholesterol, blood cholesterol, lipoproteins and atherogenesis involve multiple and diverse, yet interrelated questions. These must be addressed through a broad program of research; there is no single large-scale study to resolve these questions. A comprehensive program of research must continue and evolve to emphasize the priority areas identified--particularly points 1, 3 and 4 above--without neglecting other relevant topics.

The results of such research should have early practical application. In addition to furthering knowledge, better programs for defining persons at risk will evolve and better strategies for prevention of atherosclerosis can be developed. An important impact on the heavy burden of cardiovascular illness and death can be made.

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APPENDICES

APPENDIX A. ASSESSMENT OF THE LITERATURE

1. THE RELATIONSHIP OF DIETARY CHOLESTEROL TO SERUM CHOLESTEROL CONCENTRATION AND TO ATHEROSCLEROSIS IN MAN: TO 1979

The relationship of dietary cholesterol to serum cholesterol concentration and to atherosclerosis in man[1, 2]

Henry C. McGill, Jr.,[3] *M.D.*

Introduction

Historical background

Biochemistry of cholesterol. The discovery of cholesterol is attributed to Michel Eugéne Chevreul of France, who in 1812 first differentiated between saponifiable and nonsaponifiable lipids. Alolf Windaus of Freiburg began work on the structure of cholesterol in 1903, and Heinrich Wieland of Strasbourg began to investigate the bile acids in 1912. The correct structure of cholesterol was finally established in 1932 (1).

Early history of atherosclerosis. In 1904, Felix Marchand of Leipzig proposed the distinctive name "atherosclerosis" for the arterial lesions that had been differentiated from syphilitic aortitis, Mönkeberg's sclerosis, and arteriolar sclerosis (2). A few years later, while working on the structure of cholesterol, Windaus (3) found that the atheromatous aorta contained up to 20 times as much cholesterol and cholesterol esters as the normal aorta. Soon thereafter, James Herrick (4) of Chicago clearly identified the clinical syndrome of myocardial infarction and associated it with coronary atherosclerosis and thrombosis. However, these observers did not suggest that dietary cholesterol might be responsible in part for atherosclerosis.

Cholesterol and experimental animals. An independent line of investigation cast suspicion on dietary cholesterol as an etiological agent for atherosclerosis. In 1908, Ignatowski of St. Petersburg, investigating the effects of animal protein from meat, milk, and eggs on rabbits, observed aortic intimal lesions resembling those of human atherosclerosis. Anitschkow, also of St. Petersburg, in 1913 traced the effect of these foods to cholesterol by feeding rabbits the pure chemical dissolved in vegetable oil and reproducing identical lesions (reviewed by Anitschkow (5)). The effects of dietary cholesterol were duplicated in many other species of experimental animals. The role of dietary cholesterol in the etiology of human atherosclerosis, however, remained obscure.

Observations from the Far East. A Dutch physician, De Langen (6, 7), migrated to Java in 1914 and reported the first recorded observations on the associations between diet, serum cholesterol, and atherosclerotic disease in humans. Snapper (8), describing his experience in the Clinics of the Peiping Union Medical College in North China, commented on the rarity of coronary thrombosis, angina pectoris, and arteriosclerosis among the Chinese, even when diabetic. In speculating on the cause, he wrote, "The Chinese diet contains only small amounts of cholesterol, but considerable quantities of unsaturated acids, especially of linoleic and linolenic acid." He also mentioned the rarity of arteriosclerosis in the population of the Dutch East Indies, but pointed out that they consumed the highly saturated fatty acids of coconut oil.

Linking human atherosclerotic disease to cholesterol. The rapid increase in frequency of coronary heart disease and of other clinical manifestations of atherosclerosis in the 1940's and 1950's in the United States led to more intensive investigation of its etiology. The observation that cardiovascular disease mortality declined in areas of Western Europe

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[2] Supported in part by Grant HL-19362 from the National Heart, Lung and Blood Institute.

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during World War II when butter, eggs, and meat were scarce cast suspicion on these dietary components as possible causes of atherosclerosis. The demonstration in the early 1950's that the probability of developing coronary heart disease and other sequelae of atherosclerosis could be predicted by the concentration of cholesterol in the serum of apparently normal persons focused attention on factors that affected serum cholesterol concentration.

Saturation of dietary fatty acids and serum cholesterol concentration. The finding in 1952 that the saturation of dietary fatty acids affected serum cholesterol concentration in humans rapidly overshadowed any suspicion that dietary cholesterol was important. Although dietary cholesterol clearly elevated serum cholesterol in many animal species, its effect on serum cholesterol concentration in humans was doubted until about 1960. In controlled metabolic ward experiments, dietary cholesterol did elevate serum cholesterol concentration. Subsequently, almost all attempts to reduce serum cholesterol concentration by diet modification included reductions in dietary cholesterol.

Importance of the question

Eggs as source of dietary cholesterol. In most diets, the intakes of cholesterol and saturated fat are associated closely, and most diets designed to control hyperlipidemia include the reduction of both components. However, in formulating practical recommendations for the general public, the independent contribution of dietary cholesterol to hypercholesterolemia is important because one particular food, the egg, contributes about 46% of the total cholesterol, but only about 4% of total fat and 3% of saturated fat to the average American diet. Eggs, on the average, contribute about 39% of total cholesterol, but only about 3% of total fat and 2% of saturated fat in 20 developed countries (J. Stamler and P. Rhomberg, Northwestern University, Chicago, personal communication). Eggs, therefore, contribute few calories and little saturated fat to the diets of the Western developed countries where hypercholesterolemia is prevalent, but they contribute a large proportion of the cholesterol. Thus, the appropriateness of advocating a reduction in egg consumption to control hypercholesterolemia and thereby to prevent atherosclerosis hinges on the independent effect of dietary cholesterol. Similar questions arise with liver and other foods which are high in cholesterol but low in saturated fat. For a thorough list of the cholesterol contents of a wide variety of foods, see Feely et al. (9) and Adams (10).

Scope of this review

The lipid hypothesis. Most investigations of the role of dietary cholesterol in human atherogenesis have assumed that elevated serum cholesterol concentration is associated so closely with the progression of human atherosclerosis that it is almost certainly an intervening variable in the process. Much evidence suggests that an elevated serum concentration is essential for the other risk factors to have an appreciable effect on either arterial lesions or clinical disease. The elevation needs only to be modest to be significant in atherogenesis. This assumption is part of what commonly is referred to as the "lipid hypothesis." We will not attempt a critical review of the role of elevated serum cholesterol concentration in atherogenesis. Obviously, if it should turn out that hypercholesterolemia is *not* important in atherogenesis, much of this review would be irrelevant since very few investigations have examined directly the link between dietary cholesterol and atherosclerotic disease.

Animal experiments. The dramatic effects of dietary cholesterol in many animal species on serum cholesterol concentration and on the induction of atherosclerosis-like arterial lesions are unquestioned. They often are cited as evidence for the importance of dietary cholesterol as an etiological agent in man for hypercholesterolemia and atherosclerosis. However, there is a wide spectrum of sensitivity to dietary cholesterol among animal species, and it is not certain which species most closely approximates the sensitivity of the human. Also, most experiments have combined fat with cholesterol and few, if any, have examined the independent effects of dietary cholesterol. We will not attempt to review here the vast literature on experimental atherosclerosis.

Type of fat versus cholesterol in diet. The

effect of saturation of dietary fatty acids on serum cholesterol concentration has been demonstrated repeatedly. Although, as mentioned previously, dietary cholesterol and saturated fat usually are associated with one another in the diets of developed countries, one food, the egg, contributes much cholesterol but little saturated fat. This review, therefore, will be concerned with the evidence bearing on the effect of cholesterol *alone* on human serum cholesterol concentrations, human atherosclerotic lesions, and human atherosclerotic disease. However, there may be an interaction between dietary cholesterol and amount or type of dietary fat, and we will include in this review any evidence of such an interaction.

Types of studies reviewed. Dietary cholesterol has been implicated as a causative factor in human atherosclerotic disease on the basis of both observational and experimental studies of human groups. The observational studies range from the examination of data on the populations of entire nations or regions down to much smaller groups with distinctive eating habits. Unfortunately, as we will see, the many confounding variables that exist among such free-living groups make firm conclusions regarding dietary cholesterol difficult to achieve. We will review many of these reports because they are cited so often as evidence for the dietary cholesterol effect, but their limitations will be emphasized.

We will review all the experimental studies that we have been able to find, regardless of the number of subjects, quality of the research design, or direction of the results. Some of these, obviously, are more anecdotal than experimental, but the controversial nature of the topic demands a comprehensive examination of all recorded evidence in order to place such studies in proper perspective.

Numerous small and large controlled clinical trials of both institutionalized and free-living persons have used diets in which both dietary cholesterol and saturation of fat were lowered in order to reduce serum cholesterol concentration maximally and thereby to prevent either new or recurrent atherosclerotic disease events. No clinical trials have attempted to control hypercholesterolemia or atherosclerotic disease by reducing cholesterol intake *alone*, because all have attempted to achieve maximal reductions in serum cholesterol levels. The results of these trials shed no light (nor were they intended to) on the independent effects of dietary fat and of dietary cholesterol. Although these studies have demonstrated conclusively that dietary manipulation can reduce the average serum cholesterol concentration of a group, and have obtained suggestive evidence that such reduction is associated with reduced coronary heart disease risk, we will not review these reports because they do not help in evaluating the independent effect of dietary cholesterol.

Observational epidemiology

World War II Europe

Mortality and atherosclerosis before and during World War II. After World War II, there appeared several reports of war-time changes in mortality rates from selected diseases, particularly those related to cardiovascular disease, in the countries of Northern Europe. Vartiainen (11) compared assignments of cause of death on death registrations for two 5-year periods: 1936 to 1940 and 1941 to 1945. War-time deaths attributed to diabetes, cholecystitis, and cholelithiasis decreased, and those attributed to gastric and duodenal ulcers and to acute yellow atrophy of the liver (hepatitis) increased. Deaths attributed to "diseases of the circulatory system" remained about the same. He did not examine in greater detail the different types of circulatory system diseases, but later compared postmortem records from the years 1933 to 1938 with those from 1940 to 1946 (12). His summary states, "In each of the war-time years incidence of arteriosclerosis was found to be lower than in the corresponding peace-time year. The decrease was most marked at the age of 30–49 years and still relatively great at 50–60 years. The number of yellowish lipoid spots was identical in both materials."

Stockholm mortality before and during World War II. Henschen tabulated death rates from arteriosclerosis and "chronic myocarditis" in Stockholm during the years 1928 to 1945 ((13) cited by Malmros (14)). The mortality curve rose until 1941 but fell steeply between 1942 and 1943 and after that climbed to its former level. Henschen associated this

drop in frequency of deaths from the two causes with the rationing of foodstuffs, which in 1942 to 1943 led to reduction in the consumption of butter, eggs, and meat.

Scandinavian and United States mortality versus selected foods. Malmros (14) reviewed the relation of nutrition to health based on war-time changes in tuberculosis, diabetes, arteriosclerosis, and "cardiosclerosis" in Northern Europe. In Sweden and Finland the crude death rate from arteriosclerosis rose from 1935 until 1941. The rate began to rise again in Sweden in 1942; and in Finland, in 1943. In Norway, the rate fell steadily from 1935 until 1945, when it began to rise. In the USA, the rate rose steadily from 1935 to 1947. Malmros charted per capita consumption of total fat, eggs, and dairy products for a comparable period. Consumption of total fat in the United States remained approximately level; consumption of eggs and dairy products rose. There was a lower total fat consumption in Sweden and a 10% drop in 1941 to 1943, and a greater drop in egg and dairy products. In Norway there was a much greater drop in total fat (about 50%), and a similar large drop in eggs and dairy products during the war years. A similar marked decline in total fat consumption (to about 60% prewar level) occurred in Finland with an accompanying decline in consumption of eggs and dairy products.

Malmros discussed many of the defects in the data, such as the changing codes for classifying causes of death on death certificates, the differences in availability of food in urban versus rural areas, and other problems. However, he concluded that "the mortality from arterio- and cardiosclerosis declined in Finland, Norway, and Sweden during the 'lean' years of the war. It is clear that this is associated with a reduced consumption of eggs, butter and other foodstuffs rich in cholesterol."

Scandinavian mortality and food supplies. Biörck (15) compared mortality rates for cardiovascular disease with the differences in World War II food supplies for Denmark, Finland, Norway, and Sweden. There were numerous parallels between the severity of food rationing, the reduced consumption of meat, eggs, and butter, and reduction in cardiovascular mortality rates. These observations, he suggested, cast suspicion on food as a causative factor in arteriosclerotic disease, but did not implicate any particular food component.

Secular trends in Norwegian mortality rates. The best of this group of reports came from Strom and Jensen of Oslo (16). Using data from Norway for 1927 to 1948, they computed age adjusted and sex specific death rates, and examined in detail the effects of changes in coding procedures for causes of death during that period.

Their data showed (contrary to the data from Norway presented by Malmros) a steady rise in mortality from diseases of the circulatory system from 1927 to 1939. There was a marked drop in mortality from each of the diseases of the circulatory system and for each age group from 1939 to about 1945, when the rates began to rise again. The age adjusted death rate for circulatory diseases and the per capita consumption of fats in the form of butter, milk, cheese, and eggs were associated closely over time, with the mortality rate following fat consumption by about one year. Although comparisons of the urban-rural differences showed mixed results, the drop in mortality often occurred in rural areas as well as in the cities.

Strom's conclusion was admirable for its conciseness, its lucidity, and particularly for its restraint. He wrote, "The war-time decline in mortality . . . involved all the most important causes of death from circulatory diseases . . . [and] coincided with severe dietary restrictions. The supply of calories was reduced, and this reduction was principally of foods containing fat, including those rich in cholesterol. More definite conclusions are impossible. Our investigations have yielded, not certain proof, but several pieces of evidence for the dietary hypothesis. We have moreover, been unable to find an alternative explanation of this remarkable decline."

Critique of analysis of World War II mortality rates. It is easy to criticize the validity of the association between cardiovascular mortality and dietary restrictions in Northern Europe during World War II. Many changes other than diet were taking place. For example, there were changes in cigarette smoking habits, and these changes have not been subjected to detailed investigation and anal-

ysis. The general reduction in total caloric intake and overall loss in body weight may have ameliorated hypertension and diabetes. Probably there were other confounding environmental changes. The short latent period (1 year) makes it difficult to explain a dietary effect on atherosclerotic lesions, and suggests that the effect, if any, may have been on the terminal thrombotic episode. No tests of statistical significance were used in any of the analyses, and only the mortality rates assembled by Strom and Jensen (16) were adjusted for age and specific for sex. No data specifically implicated cholesterol as the responsible agent, but several of the investigators suggested cholesterol as one food component possibly responsible for the changes in cardiovascular mortality. Nevertheless, whether the associations with diet represented a causal relationship or whether they were coincidental, they provided a powerful impetus to the dietary fat hypothesis and stimulated the first genuine hope that atherosclerosis and its sequelae could be prevented by dietary modification.

National or regional population studies

Mortality and food consumption in 20 countries. Jolliffe and Archer (17) searched for associations between cardiovascular mortality and food consumption in 20 technically developed countries. They found strong correlations with several dietary components, particularly saturated fat, but they did not consider cholesterol independently. In their conclusion, they advised caution in the interpretation of associations based on large groups such as these because of the many intercorrelations between factors that might be thought to be independent.

Mortality and dietary cholesterol in 24 countries. Connor (18) compared death rates from arteriosclerotic heart disease among 55 to 59-year-old men, 1955 to 1956, with daily cholesterol intake estimated from food balance sheets for 1952 to 1956 reported by the Food and Agricultural Organization of the United Nations. Data were available from 24 nations, and availability was the basis of selection. Estimated daily cholesterol intake ranged from 62 mg for Ceylon to 584 mg for the United States. The coefficient of correlation between the two variables was 0.828. Partial correlation coefficients were not computed.

Trends in habits and environment and in coronary heart disease. Michaels (19) reviewed the relationship of long term trends in a number of habits and environmental variables to coronary heart disease. He noted, as had many others, the close association historically with consumption of animal fats, but did not attempt to separate cholesterol from the other components of animal fats.

Mortality and diet in 15 countries. Lopez-S. et al. (20) compared arteriosclerotic heart disease death rates and food intakes for 15 European countries between 1934 and 1959. They computed simple correlation coefficients between these death rates and a number of dietary components, but did not include dietary cholesterol. Correlation coefficients for percent calories fat and from saturated fat were high, and undoubtedly also would have been high for cholesterol had this been assessed.

Changes in mortality versus changes in diet. Masironi (21) computed correlation coefficients for arteriosclerotic heart disease death rates and a number of dietary factors in 37 countries. Death rates were correlated strongly and positively with total fat and saturated fat intake. However, *changes* in arteriosclerotic heart disease death rates were not associated with *changes* in diet. Cholesterol alone as a dietary component was not evaluated.

Mortality and egg consumption in England and Wales. Eskin (22) compared the age-specific coronary heart disease death rates for men in three age groups with egg consumption in England and Wales from 1920 to 1966. Graphically, there was a remarkable parallel between the death rates from coronary heart disease and the consumption of eggs over this 50-year period. No attempt was made to test statistically for correlation nor to correct for other associated variables.

Mortality and arteriosclerotic heart disease in 20 countries. In two reviews of the epidemiological associations between environmental factors and coronary heart disease, Stamler and associates compared dietary components with arteriosclerotic heart disease rates in 20 developed countries on a national basis

(23, 24). The correlation coefficient between daily cholesterol intake and 1964 coronary heart disease mortality rate for 45 to 54-year-old men was 0.617 ($P < 0.01$); for 55 to 64-year-old men, 0.685 ($P < 0.01$); for 45 to 54-year-old women, 0.399 (not significant); for 55 to 64-year-old women, 0.495 ($P < 0.05$). As anticipated, coronary heart disease mortality rates also were correlated to about the same degree with national income and other environmental factors such as cars per 100 persons, cigarettes consumed, calories per day, total protein, animal protein, total fat, saturated fat, and sucrose. Partial correlation coefficients were not presented.

Dietary changes in Japan. Wen and Gershoff (25) compared the dietary changes in Japan from 1950 to 1968 with concurrent changes in coronary heart disease mortality. Total daily cholesterol intake rose steadily from 93 mg/day in 1950 to 381 mg/day in 1968; total fat, from 8 to 18% of calories; and saturated fat, from 1.8 to 5.1% of total calories. The investigators used two formulas (26, 27) to estimate the contribution of these changes to serum cholesterol concentration and then used the equation derived from multivariate analysis of the Framingham data to estimate the contribution of changed serum cholesterol to increased coronary heart disease. At best, the changed diet could account for only 24% of the increase in coronary heart disease. The contribution of increased cholesterol intake *alone* was not estimated.

Mortality and food consumption in United Kingdom. Armstrong et al. (28) examined the relationship between ischemic heart disease mortality and consumption of various commodities in nine regions of England, Wales, and Scotland. Analyses were performed both for commodity consumption in the same year as the mortality rates, and for commodity consumption in the ninth previous year for the regions of the United Kingdom. Egg consumption was the major commodity contributing cholesterol to the diet. For the analysis based on the same years in England, Wales, and Scotland, the simple correlation coefficients between egg consumption and ischemic heart disease mortality were 0.90 for men and 0.89 for women ($P < 0.05$). Partial correlation coefficients controlled for flour and coffee consumption were not statistically significant. The results were similar when the commodity levels of the ninth previous year were compared with the ischemic heart disease rates. Simple correlation coefficients were 0.52 for men ($P < 0.05$) and 0.45 for women ($0.05 < P < 0.10$).

Mortality and food consumption in 30 countries. Armstrong et al. (28) performed similar analyses on data from 30 countries. The simple correlation coefficient for egg consumption and ischemic heart disease rates was 0.56 ($P < 0.05$) for men, and 0.59 for women ($P < 0.05$). As with the regional data, the value lost statistical significance when other variables (sugar, cigarette consumption) were considered in computing partial correlation coefficients.

Summary and critique of national and regional data. Despite the many comparisons of arteriosclerotic heart disease mortality among nations, states, and regions, few of these have attempted to relate mortality to dietary cholesterol or to egg consumption. The few that have done so found a strong association between mortality and cholesterol intake on the basis of simple correlation coefficients. However, when the many other interrelated variables were considered in computing partial correlation coefficients or multiple regression equations, the association with dietary cholesterol or egg consumption diminished markedly or disappeared. Therefore, these associations cannot be considered evidence for a causal relationship.

Small group studies

Observations on free-living American men. Keys et al. (29) found no difference in serum cholesterol concentrations between men who habitually consumed diets high in cholesterol and those whose diets were low in cholesterol. Forty-one normal free-living middle-aged men who voluntarily reduced their cholesterol intake by about 50% showed no change in serum cholesterol concentration after several months on the lower cholesterol intake. In contrast, two patients with familial hypercholesterolemia showed a marked drop in serum cholesterol when changed to a cholesterol-free diet. The authors concluded that the amount of cholesterol consumed daily

over about 200 mg had little or no effect on serum cholesterol, but that reduction of daily intake to zero did reduce the serum cholesterol.

Keys et al. (30) summarized a number of both observational and experimental studies of the effects of dietary cholesterol on serum cholesterol. Six surveys of 1072 Minnesota men, 18 to 58 years old, showed no association of dietary cholesterol with serum cholesterol concentration. Comparison of the serum cholesterol concentrations of 33 men who habitually consumed a low cholesterol diet (average, 401 mg/day; none greater than 600 mg/day) with those of 35 men who consumed a high-cholesterol diet (average, 1010 mg/day; all greater than 850 mg/day) showed no differences. In another 64 men who had changed their dietary cholesterol intakes (some increased, some decreased) there were no differences in serum cholesterol concentration. Addition of egg yolk to a rice-fruit diet produced no changes in serum cholesterol. Experiments with schizophrenic men given various diets showed no association of dietary cholesterol with serum cholesterol. Surveys of 105 men in Sardinia also showed no association. The authors concluded that in adult men the serum cholesterol level was "essentially independent of the cholesterol intake over the whole range of human diets."

Seven Countries Study. In the Seven Countries Study (31), extensive analyses were made of the relationship of diet and serum cholesterol concentration to the incidence of coronary heart disease but it was not possible to estimate the contribution of dietary cholesterol alone to either endpoint. The results provided strong confirmation of the association between serum cholesterol concentration and the incidence of coronary heart disease.

Vegetarians. Hardinge and Stare (32) compared the dietary cholesterol intakes of 85 vegetarians and 60 nonvegetarians with their serum cholesterol concentrations. "Pure" vegetarians consumed no cholesterol; lacto-ovo-vegetarians consumed between 300 and 600 mg/day; and the nonvegetarians consumed 600 to 1000 mg/day. The serum cholesterol levels of the pure vegetarians were significantly lower than those of the lacto-ovo-vegetarians and of the nonvegetarians. The relationship of serum cholesterol to dietary cholesterol, however, is confounded by the high proportion of vegetable fat consumed by the vegetarians and the consumption of saturated fat as well as cholesterol by the nonvegetarians.

Walden et al. (33) compared 145 healthy white Seventh-Day Adventists, who consumed a lacto-ovo-vegetarian diet, with a similar group of New York adults. Serum cholesterol concentrations were lower in the Seventh-Day Adventists, and they became even lower in subgroups that changed to a fat-restricted diet or a fat substituted (polyunsaturated fat) diet. As with other observational studies, the relationship of the difference to dietary cholesterol was confounded by other dietary and environmental differences.

Sacks et al. (34) compared plasma lipids of 73 male and 43 female vegetarians with those of age and sex matched nonvegetarian controls. Plasma total cholesterol was 58 mg/dl lower in the vegetarians, and low-density lipoproteins (LDL) cholesterol was 45 mg/dl lower. It was not possible to isolate the independent contribution of dietary cholesterol to the difference.

Critique of small group studies. In contrast to the national or regional studies, in which comparisons were made on the basis of mortality rates, small groups usually have been compared on the basis of serum cholesterol concentrations. This endpoint is necessary because of the difficulty of determining mortality or morbidity rates for such groups unless they are followed over a long period. However, in relating dietary cholesterol (or any other single food component) to serum cholesterol, the same difficulties are encountered in large or small studies—the presence of many interrelated variables which confound the results. As with the large group comparisons, we can only conclude that most results of comparison of small groups are consistent with an association between dietary cholesterol and serum cholesterol concentration, but they provide no conclusive proof of a causal relationship.

Studies based on individual values

Dietary cholesterol and familial hypercholesterolemia. Wilkinson et al. (35) collected daily diet records on 83 individuals, with and

without familial hypercholesterolemia, selected from a large kindred in which this disorder was prevalent. There was no association between the amount of cholesterol consumed per day and the concentration of serum cholesterol. No correction was made for variations in components of the diet other than cholesterol. There was no association of serum cholesterol with protein, fat, or carbohydrate content of the diet.

Dietary cholesterol in young coronary heart disease victims. Gertler et al. (36) compared the dietary cholesterol intake and serum cholesterol concentrations of 97 men who had experienced myocardial infarction before age 40 with those of 139 healthy nonhospitalized age-matched men. Nine of the coronary heart disease patients, either on physicians' advice or voluntarily, had adopted diets restricted in fat and cholesterol. Daily cholesterol intakes ranged from 86 to 1,343 mg/day as measured by dietary histories. The mean daily cholesterol intake of the coronary heart disease patients averaged slightly less than that of the controls, and their mean serum cholesterol concentration was lower. Correlation coefficients for ingested cholesterol versus serum cholesterol concentration within each group were essentially zero. Even when the extremes of serum cholesterol levels were extracted and considered separately, no association could be found between the two variables.

Serum cholesterol and dietary cholesterol in elderly persons. In 530 apparently healthy elderly men and women (all over 50 years) Gillum et al. (37) compared the serum cholesterol concentrations with dietary intakes of several major nutrients as measured by 7-day diet records. The correlation between dietary cholesterol and serum cholesterol concentration was 0.12 and was statistically significant. A similar correlation between serum cholesterol and total fat intake was found. Although the correlation coefficients were statistically significant, their magnitude suggests that neither cholesterol nor fat as estimated by 7-day diet records explained much of the variability in serum cholesterol among these elderly individuals. It is possible that many of the highly susceptible individuals had been removed from such a group by selective mortality.

Morbidity, mortality, and dietary cholesterol in Michigan men. Paul et al. (38) observed about 2000 men 40 to 55 years old for 4 years and analyzed a number of environmental factors, habits, and characteristics for relationship to one another and to coronary heart disease. Eighty-eight men developed coronary heart disease during this period. The average cholesterol intake (determined by interview) of the noncoronary subjects was 757 mg/day; that of the coronary cases was 721 mg/day. Obviously, there was no significant difference between the two groups, and there was no association of dietary cholesterol with the occurrence of coronary heart disease. The serum cholesterol concentrations of coronary subjects, however, were significantly greater than those of noncoronary subjects. Note that, in this study, diet was assessed before coronary heart disease developed, in contrast with the study of Gertler et al. (36).

Serum cholesterol and dietary cholesterol in British men. Morris et al. (39) studied intensively 99 British bank employees ages 40 to 55. Diets were assessed by weighing food consumed. There was a wide range in fat and cholesterol intake and also in serum cholesterol concentration (154 to 324 mg/dl). No association could be found between *any* dietary variable, including cholesterol, animal fat, total fat, or ratio of saturated to polyunsaturated fat, and serum cholesterol concentrations.

Serum cholesterol and dietary cholesterol in Israeli men. Kahn et al. (40) examined the relationship of serum cholesterol to dietary cholesterol and a number of other variables in 10,000 Israeli men of diverse origins. There were wide ranges in dietary cholesterol intake (as measured by diet history) and in serum cholesterol concentrations. No simple correlation coefficients between *individual* values of any variables versus serum cholesterol were appreciable in magnitude. In regression equations, the best linear combination of variables was associated with only 10% of the variance in serum cholesterol concentration. The authors believed that these results did not necessarily contradict the numerous experimental observations showing an effect of cholesterol and fat on serum cholesterol. They suggested that diet is only one of many factors affecting serum cholesterol; and therefore, when other conditions are not kept con-

stant, the diet (including dietary cholesterol) contributes only a small proportion of the variance.

Serum cholesterol and dietary cholesterol in the Framingham Study. The Framingham Study (41) examined the relationship of cholesterol intake to serum cholesterol in 912 subjects on the 5th biennial examination (1957 to 1960). Men consumed, on the average, 704 mg cholesterol per day (range about 250 to 1500 mg/day); and women, 492 mg/day (range about 150 to 1300 mg/day). There was no association between cholesterol intake and serum cholesterol levels.

Serum cholesterol and dietary cholesterol in children. Hodgson et al. (42) compared the dietary intakes of 29 children between 7 and 12 years of age with their serum cholesterol concentrations. Dietary cholesterol intake ranged from 150 to 394 mg/day. There was no correlation between cholesterol intake and concentration of serum cholesterol.

Frank et al. (43) compared dietary cholesterol intake and serum cholesterol concentration in 185 children 9 to 11 years old. Dietary intake estimated by 24-hr recall ranged from none to 1536 mg/day; mean, 324 mg/day. Converted to mg/1000 kcal, the mean cholesterol intake was 150. Eggs contributed 26 percent of the cholesterol; milk, 16%; and beef, 13%. Dietary cholesterol had a weak positive correlation coefficient with β-lipoprotein concentration (0.171, $P < 0.05$), but the correlations with total serum cholesterol and other lipoprotein values were not significant. Partial correlation coefficients controlling for other variables were not computed.

Coronary atherosclerosis and dietary cholesterol. Moore et al. (44) conducted a unique study in which they obtained dietary histories for 253 deceased New Orleans men 20 to 60 years of age. The histories were obtained by interviewing surviving women who had shared the household with the men for an average of 18 years. Each respondent reported, by a detailed questionnaire, the usual 28-day pattern of food intake of the subject during the terminal year of his life. The extent of advanced atherosclerotic lesions (raised lesions) was estimated in the coronary arteries of each subject by a team of pathologists.

The cases were grouped into tertiles by mean daily dietary intakes and also by mean daily nutrient/calorie ratios. Cholesterol intake ranged from an average of 465 mg/day for fifteen 20 to 24-year-old men to 1333 mg/day for fifty-two 55 to 60-year-old men. The means of extent of raised lesions in the coronary arteries of the subjects for each tertile were then analyzed for differences and trends by analysis of variance. The results indicated that higher daily intakes of protein of vegetal origin, total carbohydrate, starch, and crude fiber were associated with *less* extensive atherosclerotic lesions. There were no associations between daily intakes of other nutrients and atherosclerotic lesions.

When the results were examined on the basis of nutrient/calorie ratios, starch and vegetal protein were associated with less atherosclerotic lesions, while animal protein and fat, regardless of source, were associated with greater atherosclerotic lesion involvement. No associations of dietary cholesterol with lesions were found either on the basis of total daily intake or mg/1000 kcal.

Serum cholesterol and dietary cholesterol in Tecumseh Study. Nichols et al. (45) conducted 24-hr dietary recall interviews among 957 men and 1,082 women 16 through 69 years of age in Tecumseh, Mich. Subjects were classified according to the deviation of observed serum cholesterol values (performed during the 1967 to 1969 examinations) from the age regression lines to remove the effects of age, and were grouped into tertiles within each sex. The overall mean daily cholesterol intake of men was 551 mg; of women, 328 mg; and there was a wide (but unspecified) range of individual intakes. Among the six tertiles grouped by serum cholesterol concentration, there were no differences in mean cholesterol intake in either men or women. The authors comment, however, that the "findings do not exclude a relationship between consumption of dietary cholesterol and serum cholesterol concentration," and cite the evidence of a plateau effect at about 400 mg/day as the most probable reason for the lack of an association.

Serum cholesterol and dietary cholesterol in Puerto Rican men. Garcia-Palmieri et al. (46) compared serum cholesterol concentrations and a variety of nutrient intakes based on 24-hr recalls in about 2500 rural and 6000 urban Puerto Rican men. Correlation coefficients

and regression coefficients were computed within each group. No correlation coefficient was greater than 0.15. Among the regression coefficients, those for mg cholesterol intake per day were positive and significant but very small (0.0078, age 45 to 54; 0.0124, age 55 to 64) for urban men, and small and not significant for rural men.

Serum cholesterol and dietary cholesterol in Tarahumara Indians. Connor et al. (47) investigated the relationships between serum lipid concentrations and various dietary components in the Tarahumara Indians, a tribe of the Sierra Madre Occidental Mountains of Mexico. These Indians are not acculturated to Western habits and are noted for their extremes of physical activity. Among one hundred forty-nine 19 to 70-year-old men, the mean plasma cholesterol concentration was 136 mg/dl; in 108 women of similar ages, it was 139 mg/dl. Only 4% of the adults (excluding pregnant and lactating women) had plasma cholesterol levels over 180 mg/dl. About 66% of the total plasma cholesterol was in LDL, and about 18% was in high-density lipoproteins (HDL). The average daily intake of cholesterol was 71 mg for adult men and 75 mg for women; for children, it was about 33 mg/day. Total fat intake was 38 g/day for men and 28 g/day for women, providing 11 to 12% of calories from fat; and only about 20% of the fat was saturated.

The correlation between plasma cholesterol concentration and dietary cholesterol intake in 103 Tarahumara Indian adults was 0.898. The range of cholesterol intakes in this subsample was 17 to 144 mg/day; the range of plasma cholesterol concentrations was about 80 to 230 mg/dl. There also were positive and significant correlations between plasma cholesterol concentrations and animal fat (0.593), total fat (0.552), animal protein (0.464), sugar (0.323), and eggs (0.548). Eggs contributed about 70% of the dietary cholesterol. The authors emphasized that cholesterol intake probably was strongly correlated with plasma cholesterol concentration because the cholesterol intakes were in the range of a linear response (i.e., from 0 to about 300 to 400 mg/day). The authors did not present partial correlation coefficients to assess the unique contribution of dietary cholesterol to plasma cholesterol, nor did they analyze the data for interactions with fat. The possibility remains that the correlation coefficient might be reduced considerably if the intercorrelations among the other dietary factors were taken into account, as in computation of a partial correlation coefficient.

Coronary heart disease and dietary cholesterol in Honolulu. Yano et al. (48) assessed nutrient intakes of 7705 healthy men in the Honolulu Heart Study by 24-hr recall interviews at the time of initial examination (1965 to 1968). During a 6-year follow-up period, 294 new coronary heart disease cases occurred among the subjects. The mean daily intake of cholesterol was 549 mg (SD, 318) for the men who did not develop coronary heart disease; and 521 mg (SD, 293), 557 mg (SD, 330), and 587 mg (SD, 312) for men with three different types of coronary heart disease. Obviously, the differences were not significant, in spite of a previous finding that the level of serum cholesterol had been identified as an important risk factor for coronary heart disease in this same population (49). Furthermore, there were no differences between cases and noncases in intake of saturated fat or of unsaturated fat.

The authors discussed in considerable detail the possible reasons for the lack of dietary differences between cases and noncases, a finding that was contrary to prevailing ideas of the dietary etiology of atherosclerosis. They attributed the failure to the high degree of variability among individuals within the culturally homogeneous population, error in the 24-hr dietary recall method, and changes in dietary habits over a lifetime.

Critique of studies based on individual values. These studies usually have tested associations between dietary cholesterol and serum cholesterol concentration, but three tested the association of dietary cholesterol with coronary heart disease incidence (case-control, (36); longitudinal, (38, 48)), and one tested the association with coronary artery atherosclerotic lesions (44). The results, with one exception, yield very weak or no associations. In the one exception of the Tarahumara Indians (47), the low range of cholesterol intake may explain why an association is present here but not in other populations; but these results cannot be accepted as conclusive evidence of an independent cholesterol effect

unless the association can be shown to be independent of the other interrelated variables.

Thus, in considering the results of studies based on individual values, the issue is the reverse of that with group data—there is little or no association of dietary cholesterol with either serum cholesterol concentration, coronary atherosclerosis, or coronary heart disease. The following sections examine some of the reasons for this discrepancy and its implications for the role of dietary cholesterol as a contributing cause of atherosclerotic disease.

The problem of correlations in observational studies

The problem. A striking feature of the results obtained in the observational studies reviewed in the preceding sections is the contrast between the high simple correlation coefficients obtained from group averages or rates and the low correlation coefficients obtained with individual values. In both types of studies, the coefficient of correlation was greatly reduced when other associated and interdependent variables were taken into account by computing partial correlation coefficients. The absence of strong independent associations of dietary cholesterol with serum cholesterol concentrations or mortality rates in larger group comparisons, and the low or zero correlation coefficients for dietary cholesterol and serum cholesterol concentrations or incidence rates in individual studies often have been cited as evidence that dietary cholesterol does not affect serum cholesterol concentration and does not contribute to the etiology of atherosclerotic disease. Three separate problems are involved here: *1*) the confounding of all observational studies by multiple interdependent variables; *2*) the subtle but fundamental difference between group correlations and individual correlations; and *3*) the effects of within and between subject variation and methodological error on the suspected relationship.

Confounding variables. It is almost impossible to find a substantial number of individuals who consume a high cholesterol, low saturated fatty acid diet. As noted in the introduction, eggs contain much cholesterol but little fat; but egg consumption, the richest single source of dietary cholesterol, is closely associated with the consumption of animal fats and dairy products that are rich in saturated fatty acids.

Furthermore, the high cholesterol, high saturated fatty acid diet is typical of affluent groups which often have a high prevalence of other characteristics (such as cigarette smoking, obesity, diabetes mellitus, physical inactivity) known to influence serum lipids and atherosclerotic disease. Consequently, we can only repeat the cautions expressed by other investigators (for example, Jolliffe and Archer (17)) regarding the interpretation of associations based on observational studies, particularly those of large groups.

Group versus individual correlations. There is a fundamental difference between correlations among group averages or rates, and those among individual values. Robinson (50) contrasted the underlying assumptions and the significance of ecological correlations (based on group values) with those of individual correlations, and stated mathematically the exact relation between the two. Both by mathematical analysis and by examples, he showed that the correlation based on individuals within each group may be negative, and yet the ecological correlation may be positive. The ecological correlation is determined by marginal totals of the groups included, and not by the individual correlations within each group. Robinson concluded that ecological correlations *cannot* be used as substitutes for individual correlations. Theoretically, they can be equal, but the conditions under which this can occur seldom exist.

This fundamental difference between group and individual correlations may be a major reason for the discrepancy between the two types of correlations for dietary cholesterol and either serum cholesterol or arteriosclerotic heart disease. We must interpret the group correlations as describing groups, and not describing the responses of individuals to specific environmental agents. The positive correlation coefficients between dietary cholesterol and various endpoints on the basis of group values can be interpreted only as suggesting an association.

Effects of intraindividual variation and methodological error. Liu et al. (51) examined

the roles of intra-individual variation and measurement error on correlations between dietary intakes and serum cholesterol concentrations. The estimated correlation between two variables, as customarily computed, is much less than the true correlation if intraindividual variation is large for only one variable. Obviously, if both variables exhibit large intraindividual variation, the correlation is degraded even more. The large day-to-day variability in serum cholesterol concentration is well known, and likewise there is a large variability in dietary intake patterns of most persons. The individuals in the study sample must be followed for a sufficiently long time for both diet and serum cholesterol to allow a reliable estimate of the true mean for each individual. Increased numbers of measurements do not reduce the day-to-day variation within subjects, but they permit a more accurate estimation of the parameters in question for each individual.

Using actual daily food intake records, Liu et al. (51) computed the numbers of days that food records would have to be taken to reduce the error term for the correlation coefficient to an acceptable level. In the instances presented, records would be required for 7 to 10 days, longer than most such studies have used. Similar considerations apply to the number of serum cholesterol determinations required to reduce the error caused by day-to-day variation.

Jacobs et al. (52) suggested that the most important condition that prevents finding an association, even though a relationship might exist, is the nature of the serum cholesterol concentration itself. There is variability among persons in serum cholesterol concentration even under the least cholesterolemic dietary conditions (the "intrinsic level"). To this is added variability in response to cholesterolemic dietary components. Neither the intrinsic level nor the response can be measured in a cross-sectional study; only the final serum cholesterol concentration, which is the sum of the intrinsic level and the response, will be known. The coefficient of correlation is quite sensitive to individual variability, and a true relationship may be obscured easily under these circumstances.

Using data from an intervention trial in which the response and the intrinsic level were estimated in a small group, Jacobs et al. (52) found a correlation coefficient between a dietary "score" and serum cholesterol *change* (response) of 0.4, considerably higher than other studies using conventional cross-sectional techniques. The dietary score involved dietary components other than cholesterol, but their analysis illustrates the principle. Measuring the response to a *change* in diet is the objective of the experimental studies that are reviewed in the next major section.

From these considerations, it is apparent that the zero correlations obtained in many cross-sectional studies within populations do not necessarily negate the existence of a real relationship in the population. Indeed, from the known variability of both dietary intake and serum cholesterol concentration, one can predict that a low correlation would be found on the basis of a single serum cholesterol determination and a one day diet record, even if there were a strong effect of diet on serum cholesterol concentration. The test of a relationship between dietary cholesterol and serum cholesterol is much more sensitive if the same individuals are examined with and without exposure to dietary cholesterol.

Experimental studies in humans

Introduction

This section reviews those reports in which only dietary cholesterol was treated as an independent variable, or in which dietary cholesterol and another dietary variable were manipulated in such a manner that the independent effect of cholesterol on serum cholesterol concentration could be assessed. In comparison with the vast literature concerned with the relationship of diet to serum cholesterol and lipoprotein concentrations, lipid metabolism, or coronary heart disease, this collection may seem to represent pitifully little data. Experimentation with the independent effect of dietary cholesterol in humans has been overshadowed by the heavy emphasis on type of fat. Experiments and clinical trials that have manipulated dietary cholesterol and type and amount of fat in attempting to achieve a maximal reduction in serum cholesterol concentration provide no information on the contribution of dietary choles-

terol to serum cholesterol concentration. Neither do they provide information on the degree to which removal of cholesterol contributes to any observed reduction in clinical disease manifestations.

Before 1950

Except for the paper by De Langen (7) which lay unnoticed for many years, only four reports of experiments concerned with this issue before 1950 could be found. The differences among these four predict rather well the controversy that followed.

Lack of response in young women. Okey and Stewart (53) fed four normal women students a standardized diet to which egg yolk and liver were added to provide 3100 mg cholesterol, and the same diet with a similar amount of cholesterol dissolved in butter. The feeding periods were 4 weeks. The average increase in serum cholesterol in the four subjects on the egg yolk supplemented diet was 13 mg/dl; on the cholesterol supplemented diet, 5 mg/dl. The variability in individual response was so great that no overall conclusion regarding dietary cholesterol effects was possible.

Response in hospitalized patients. Steiner and Domanski (54) fed 10 hospitalized patients (eight with rheumatoid arthritis, two with chronic nephritis) a dietary supplement of 100 g/day of dried egg yolk powder for 6 to 10 weeks. On the average, serum cholesterol concentration increased 101 mg/dl, with a range of 50 to 218 mg/dl. After the egg yolk supplement was discontinued, the serum cholesterol concentration returned in a few weeks to a relatively constant baseline level of 228 mg/dl.

Lack of immediate response in children. Heymann and Rack (55) gave various amounts of cholesterol dissolved in olive oil to several infants, children, and adults. The doses ranged from 70 to 670 mg/kg body weight. They found no increase in the concentration of serum cholesterol within 48 hr of a single dose of cholesterol in one group of subjects, and no increase over several weeks in another group of subjects who received cholesterol daily.

Immediate response in adults. Collen et al. (56) devised a cholesterol tolerance test using 250 g of fresh egg yolks (about 5000 mg of cholesterol), and measured plasma cholesterol 2 and 4 hr later. Of 27 normal adult males between 23 and 90 years, 22 (81%) had a fasting cholesterol level less than 300 mg/dl, and an increase in plasma cholesterol less than 30 mg/dl. Of 53 men who had recovered from a myocardial infarct, 43 (81%) had either fasting cholesterol levels greater than 300 mg/dl, or an increase after the ingestion of egg yolk which exceeded 30 mg/dl.

1950 to 1959

Increased investigation of dietary effects. This decade witnessed the surge of interest in coronary heart disease and the underlying process of atherosclerosis as cardiovascular mortality rates in the technically developed Western countries continued to increase. The observations that cardiovascular mortality had declined in the countries of Western Europe during World War II had focused attention on the possible dietary etiology of atherosclerosis. A great deal of animal experimentation was begun, and, for the most part, it was directed toward finding the mechanism of action of the high fat, high cholesterol diet initially used by Anitschkow.

The results of the Framingham Study and other longitudinal epidemiologic studies, which began to appear about the middle of the decade, confirmed the case-control observations that elevated serum cholesterol concentration was associated with risk of developing coronary heart disease. Attention then was directed at factors controlling this easily observed variable. Early in this decade, the cholesterol-lowering effect of unsaturated fat was discovered, and the attention of most investigators became focused on that issue.

Moderate response in middle aged patients. Messinger et al. (57) reported experiments performed in 1942 in which 19 middle-aged subjects with various diseases were fed dietary supplements of 100 to 150 g of egg yolk powder (providing 2500 to 3750 mg cholesterol) daily for up to 48 days. Total serum cholesterol concentration increased by 12 to 71 mg/dl at the end of the feeding period. The same paper reported experiments conducted in 1947 in which five hospitalized men received, at different times, up to 30,000 mg cholesterol suspended in milk or cream, and

150 g of egg yolk powder (about 3750 mg cholesterol) per day. The egg yolk powder produced higher elevations of serum cholesterol concentration than did the much larger amounts of pure cholesterol.

Response to egg yolk but not to cholesterol. Kinsell et al. (58) described the results of a number of experiments involving fat-manipulated diets in unspecified subjects. As much as 60,000 mg cholesterol daily added to a diet high in vegetable oil did not influence the serum cholesterol concentration. When egg yolk was used as the source of fat, providing about 9,600 mg of cholesterol per day, the serum cholesterol rose moderately.

Lack of response in young pregnant women. Moses et al. (59) measured the self-selected diets of 65 young pregnant women and gave 30 of them 2000 mg of cholesterol daily in chocolate candies. The average serum cholesterol concentration of the control group at the start of the experiment was 33 mg/dl less than that of the cholesterol-supplemented group. The cholesterol supplemented group spontaneously consumed less fat and calories than did the control group. The serum cholesterol concentrations of the cholesterol-supplemented group were consistently lower between the 5th and 9th months of pregnancy, but the differences were not statistically significant.

Lack of response in physicians. Mayer et al. (60) fed five male physicians and interns test diets of regular foods selected to provide different combinations of cholesterol intake (100 to 800 mg/day) and different types and amounts of fat (animal and vegetable fat, 10 to 46% of total calories). Test periods were one to four weeks. Cholesterol was derived from egg yolk. A low fat, low cholesterol diet reduced the serum cholesterol level from the control period on a regular diet, but the addition of egg yolk did not cause an increase within one week. A high fat diet containing about 100 mg cholesterol per day increased serum cholesterol concentration to about the same degree as a high fat diet with 300 mg of cholesterol per day.

Lack of response to cholesterol in formula diet. Beveridge et al. (61) studied five male faculty members, 33 to 41 years of age, who consumed alternately free choice diets and formula diets containing varying amounts of cholesterol and different types of fat for 11 days. Added cholesterol up to 200 mg/950 kcal failed to affect the serum cholesterol concentration, although butterfat elevated serum cholesterol and vegetable fat lowered it.

Lack of response in schizophrenic subjects. As part of a longer report describing observational studies on the association of dietary cholesterol with serum cholesterol concentration, Keys et al. (30) described the results of experiments with 27 male schizophrenic subjects receiving diets for 4 weeks with either 374 or 1369 mg/day. There were essentially no changes in serum cholesterol concentration related to the two experimental diets in comparison with one another or in comparison with the regular hospital diet.

Difference in response among Bantu. In a series of varied experiments with two normocholesterolemic volunteers, Bronte-Stewart et al. (62) found that both egg yolk and cholesterol added to the diet increased the serum cholesterol concentration in one 65-year-old Bantu male. However, in another 37-year-old Bantu male, the serum cholesterol concentration remained level or declined on the same intake of 10 eggs daily.

Lack of response in hyperlipidemic subjects. Ahrens et al. (63), in a review of their work on fatty acid effects on serum cholesterol, found that a large amount of cholesterol (up to 8000 mg/day) given to a hyperlipidemic patient elevated serum cholesterol concentration from about 180 mg/dl to about 250 mg/dl (read from Fig. 3 of their report).

Experiments with modified egg yolk. Horlick and O'Neil (64), in a preliminary communication, described the effects of egg yolk from hens fed large proportions of sunflower seed oil. Their yolks showed a 6-fold increase in linoleic acid at the expense of oleic acid. In two subjects fed five eggs per day for three days and 10 eggs per day for 11 days after having been fed a low fat diet for 10 days, the serum cholesterol level continued to fall on the experimental egg diet in one subject and rose to a point below the pre-experimental level in the other subject. In a later test with regular eggs, the second subject showed a substantial rise in serum cholesterol. A final report of this work (65) described marked elevations in serum cholesterol concentration in five subjects (medical students) when con-

suming large amounts of egg yolk, both regular and unsaturated.

Gordon et al. (66) also tested eggs from hens that had been fed sunflower seed to increase the unsaturated fatty acid content of their yolks. In one young male Bantu subject who had been maintained on a very low fat diet, feeding 10 regular egg yolks per day increased the serum cholesterol concentration by about 35 mg/dl, and feeding the special eggs did not significantly reduce it. In another similar subject, feeding the experimental eggs also increased the serum cholesterol concentration by about 30 mg/dl, but feeding 3000 mg of pure cholesterol daily allowed it to drop back to the previous baseline level.

Interaction between cholesterol and type of fat. Beveridge et al. (67) studied the responses of university students to cholesterol in formula diets for 8-day periods. Fifty-four men and 20 women were fed eight different diets in groups of eight to 11 individuals. The diets contained various butter oil fractions, medium chain triglycerides, and coconut oil to provide 30% of calories, with (1290 mg/950 kcal) and without cholesterol. All the fat supplemented diets except those containing medium chain triglycerides produced significant elevations in serum cholesterol concentration after 8 days. The butter-fraction diets to which cholesterol was added produced considerably higher elevations than did the diets with the fat supplement alone. However, cholesterol added to medium chain triglycerides had no effect on serum cholesterol concentration. This is one of the few studies in which an interaction between the type of dietary fat and the cholesterol in influencing serum cholesterol was shown.

Summary and critique of results of 1950 to 1959. The studies of this period yielded mixed results. Out of 12 reports which included 212 subjects, many of them patients hospitalized for various diseases or subjects with hyperlipidemia, seven experiments found an increase in serum cholesterol concentration after increasing dietary cholesterol intake. Of the two larger studies which included 139 of the 212 subjects, there was an increase in average serum cholesterol after cholesterol feeding in one group (67) and there was no change in the other group (59). There was a hint of an interaction between dietary cholesterol and saturated fat in one experiment (67). Most of the other studies are not interpretable because of small and highly selected groups of subjects or confounding by variations in amount or type of fat. There may have been problems with the precision and accuracy of serum cholesterol determinations in many laboratories of that period. The positive results of cholesterol feeding suggested that there probably was a relationship, but the relationship was weak and varied greatly among individuals.

1960 to 1969

Increased investigation of dietary cholesterol. This decade was the "golden age" of experimentation with the effects of dietary cholesterol on atherosclerosis and serum cholesterol concentration. More reports appeared describing an effect of dietary cholesterol on serum cholesterol concentration, and several studies included larger numbers of subjects and better control of other dietary components, particularly fat.

Response during metabolic study. In experiments designed primarily to determine the proportion of cholesterol in serum that was derived from dietary cholesterol, Taylor et al. (68) fed 11 human subjects up to 4000 mg of cholesterol per day as egg yolk and reported that "Most of the subjects . . . showed a serum cholesterol elevation of 10 to 40 mg%."

Response to cholesterol in young persons. Beveridge et al. (69) placed 67 university students (53 men and 14 women; ages not specified) on a homogenized, fat-free diet for 8 days. The mean serum cholesterol concentration fell from 201 to 146 mg/dl during this period. They then received diets enriched by butter oil stripped of cholesterol to provide 30% of calories from fat; on this diet, the mean serum cholesterol concentration rose to 171 mg/dl. The students were then divided into 8 groups and purified crystalline cholesterol dissolved in the butter oil was added in amounts ranging from none to 4,500 or to about 1700 mg/1000 kcal. At the end of 8 days, serum cholesterol concentration related to addition of cholesterol increased up to 34 mg/dl. The increment, expressed in average percent change in serum cholesterol concentration, was approximately linear up to a

daily intake of 634 mg/day, after which there was no further response.

Response to cholesterol under metabolic ward control. Connor et al. (70) studied six male prisoners between 40 and 45 years old, one of whom had hypercholesterolemia (280 mg/dl). For 35 days, all subjects consumed a regular diet that was estimated to contain about 40% of calories from fat and 900 to 950 mg cholesterol. For the next 75 days, three subjects received a formula diet made with peanut oil and egg yolk. One subject received 1,650 mg of cholesterol per day; the second, 1900 mg/day; and the third, 4800 mg/day. During the same period, the other three subjects received a similar formula containing no egg yolk but with equivalent fat provided by peanut oil. During this period, the total serum cholesterol varied considerably, but at the end of the 75 days, it had risen in the first subject by 36 mg/dl; in the second by 25 mg/dl; and in the third by 30 mg/dl. In the three subjects on a cholesterol-free diet, the serum cholesterol concentration fell by 61, 44, and 84 mg/dl.

During the third period of 23 days, the first group was changed to a cholesterol-free diet and the second group to a similar diet with 2,400 mg cholesterol added in the form of egg yolk. Serum cholesterol concentrations in the first group declined by 94, 115, and 98 mg/dl; and rose in the second group by 80, 15, and 76 mg/dl. These daily intakes of cholesterol were so far in excess of the usual daily intake, and the nunbers of subjects were so small, that computation of a dose-response relationship was not possible. The day to day variation within subjects and the variation in responses among subjects were considerable. The authors concluded that, contrary to most previous reports, dietary cholesterol did indeed contribute to elevation in serum cholesterol concentration.

In a more thorough and better designed follow-up of their previous demonstration of the effects of dietary cholesterol, Connor et al. (71) gave six healthy male prison inmates, 41 to 52 years, in sequence a cholesterol-free diet, a diet containing cholesterol from egg yolk, a cholesterol-free diet, and a similar diet with added crystalline cholesterol. Each diet was fed for 3 weeks. The egg yolk supplemented diet provided two men with 475 mg of cholesterol per day; two with 950 mg/day, and two with 1425 mg/day. In the cholesterol supplemented diet, the pairs received 1200, 2400, and 3600 mg/day. In each diet, a vegetable oil mixture containing about 25% polyunsaturated fatty acids provided 40% of the calories. The different amounts of egg yolk cholesterol led to similar increases in serum cholesterol concentration, on the average 69 mg/dl. The different amounts of crystalline cholesterol also produced similar elevations in serum cholesterol concentration, on the average 19 mg/dl.

In a third metabolic ward study, Connor et al. (72) investigated the interrelationships of dietary cholesterol and type of fat. Six men 24 to 48 years were the subjects. Three were prison inmates and three were juvenile diabetics receiving insulin. Mixed natural foods were combined to provide a high cholesterol (729 mg/day, 259 mg/1000 kcal), saturated fat (9.5% polyunsaturated fatty acids; P/S = 0.24) diet; a no-cholesterol, saturated fat diet, a no-cholesterol, unsaturated fat diet (31% polyunsaturated fatty acids, P/S = 1.69); and a high cholesterol (725 mg/day), unsaturated fat diet. Total fat contributed 40% of calories throughout.

The serum cholesterol concentrations of all subjects declined (average, 38 mg/dl, $P < 0.001$) when cholesterol was removed from the saturated fat diet. Serum cholesterol did not change when the fat was changed to the no-cholesterol, unsaturated fat diet. The addition of cholesterol to the unsaturated fat diet led to a rise in serum cholesterol in all subjects (average, 28 mg/dl, $P < 0.02$). Both normal and diabetic subjects had similar responses. The authors interpreted these results to indicate that the hypocholesterolemic effect of polyunsaturated fats depended on the presence of some cholesterol in the diet, and that dietary cholesterol had a major role in causing hypercholesterolemia in humans.

From the detailed data presented in this report, it is possible to rearrange the results in a format which shows the independent effects of cholesterol and saturated fat more clearly (Table 1).

The effect of adding about 725 mg of cholesterol per day of cholesterol was much greater than the effect of type of fat. From inspection of the individual values, all the

TABLE 1
Mean serum cholesterol concentrations of six subjects on four diets (72)

		Type of fat		Difference
		Unsaturated	Saturated	
Cholesterol	None	174	175	+1
	High	202	213	+11
Difference		+28	+38	

subjects responded to dietary cholesterol. The mean elevations by subjects across both types of fat ranged from 21 to 49 mg/dl. Our analysis of variance of the reported data shows a significant independent effect of cholesterol ($P < 0.005$), but no significant effect of type of fat and no significant interaction.

Miscellaneous studies. In an abstract with only limited details, Anderson et al. (73) reported moderate increases in serum cholesterol concentration with daily cholesterol intakes of 50 to 1500 mg/day. The effect of egg yolk was similar to an equivalent amount of crystalline cholesterol. They concluded that the serum cholesterol concentration was a linear function of the square root of the daily cholesterol intake.

Steiner et al. (74) fed 8 hospitalized patients (six men and two women, 50 to 63 years old, with atherosclerotic diseases and hypertension) formula diets in which olive oil provided about 40% of the calories. The test period was 4 weeks. The addition of 3,000 mg of crystalline cholesterol to the olive oil was followed by substantial rises in serum cholesterol concentration ranging from 60 to 136 mg/dl; average, 78 mg/dl.

Wells and Bronte-Stewart (75) performed numerous experiments with three male subjects, a hospital patient age 46 and two healthy volunteers. When egg yolk was added to a basic diet nearly free of cholesterol in an amount equal to 53 g of egg yolk lipid, serum cholesterol regularly and repeatedly was elevated in one subject by about 60 mg/dl. The effect was duplicated by feeding an equivalent amount of cholesterol in oils with an iodine number similar to that of egg yolk lipid. The elevation in serum cholesterol was linearly related to the log of the dose of cholesterol up to about 500 mg/day.

Response to cholesterol among Guatemalan children. Mendez et al. (76) fed cottonseed oil with and without crystalline cholesterol as a supplement to the regular diet of 60 school-age Mayan Indian children. Even with the oil supplement, only 20% of the calories were derived from fat. Neither 600 nor 1200 mg of cholesterol during two consecutive 30-day periods significantly increased serum cholesterol concentration, which remained at about 135 mg/dl in both cholesterol fed and control groups.

Mendez (77) expanded the experiments with dietary cholesterol in rural Guatemalan Indian children. These children, who were living on a low protein, low calorie, low fat, and very low cholesterol diet, had serum cholesterol concentrations averaging about 130 mg/dl, considerably lower than upper-income Guatemalan children of similar ethnic background. One group of six boys and 17 girls, average age 11.6 years, received an additional two eggs (about 600 mg of cholesterol) per day; a second group of 18 boys and three girls, average age 10.6 years, received 600 mg of cholesterol dissolved in 30 ml cottonseed oil; and a third group of 19 boys and 15 girls, average age 9.4 years, received only the same amount of oil daily. The mean serum cholesterol concentration in the group receiving eggs increased after four weeks from 120 mg/dl to 131 mg/dl ($P < 0.01$). It decreased in the group receiving crystalline cholesterol from 128 to 119 mg/dl (not significant), and also decreased in the group receiving oil alone from 136 to 127 mg/dl ($P < 0.05$).

Response to dietary cholesterol and trans fatty acids. Erickson et al. (78) fed 42 healthy male prison inmates, age 29 to 45, formula diets containing two different fat mixtures of similar fatty acid composition (P/S about 1.5 to 1.6), but with differing proportions of trans fatty acids, with and without added egg yolk powder to provide 742 mg/day per subject. These diets were part of a larger experiment involving other fats which were given seven groups of six men each in a seven-treatment, four-replication incomplete Latin square design. Four groups received each of the two diets with cholesterol and four received each diet without cholesterol, each for 5 weeks. Under these conditions, the added egg yolk increased serum cholesterol concentration by about 25 or about 8 mg/dl per 100 mg of dietary cholesterol per 1000 kcal.

Equation to predict dietary cholesterol effect. Grande et al. (79) studied the effects of various levels of cholesterol intake combined with varying degrees of saturation of fat, and also compared crystalline cholesterol with egg yolk as a source of cholesterol. The subjects were physically healthy schizophrenic men 35 to 65 years old. Ordinary foods were selected to provide a low-fat, low-cholesterol diet, to which supplements of fat, cholesterol or carbohydrate were added. Total caloric intake ranged from 2600 to 2900 kcal/day. The experimental design was of the crossover type, with 3-week dietary exposure periods for each diet.

With an unsaturated fat supplement providing about 45% of total calories, crystalline cholesterol up to 560 mg/1000 kcal produced a consistent elevation in serum cholesterol which was predicted by the equation

$$\Delta \text{ Chol} = 1.5(Z_2 - Z_1)$$

where Δ Chol was the change in serum cholesterol in mg/dl; Z_2 was the square root of the new cholesterol intake in mg/1000 kcal; and Z_1 was the square root of the previous cholesterol intake. This formula was derived from data obtained from this experiment plus data from four other reports (54, 69, 72, 78).

When cholesterol was added to saturated and to unsaturated fat supplements providing about 41% of calories and to diets in which fat was reduced to about 8% of total calories, the formula described above predicted well the absolute levels of serum cholesterol. Thus, there was no apparent interaction between cholesterol and *type* or *amount* of fat.

In one experiment, the investigators compared egg yolk cholesterol with crystalline cholesterol. Eggs produced slightly higher increments of serum cholesterol concentration than crystalline cholesterol per milligram of cholesterol, but the difference was not statistically significant.

In summary, these investigators concluded that, within the range of about 20 to 550 mg of cholesterol per 1000 kcal, the change in serum cholesterol concentration was proportional to the difference between the square roots of cholesterol intakes. The relationship was derived by comparing the effects of increases in dietary cholesterol, and not from comparing the effects of decreases in dietary cholesterol. The effect was independent of the type of fat (saturated or unsaturated) and of the amount of fat between about 8 and 40% of calories from fat.

Keys et al. (27) followed serum cholesterol changes in 22 physically healthy schizophrenic men on dietary intakes of 50 to 1460 mg/day. The diet was constant in all other respects. The source of the cholesterol was not specified, but presumably it was crystalline cholesterol since egg yolk would have complicated the maintenance of identical fat composition among the experimental diets. These diets produced changes in serum cholesterol concentration of from 5 to 11 mg/dl for each 100 mg of cholesterol per 1000 Kcal. In this paper, the same equation is presented relating dietary cholesterol to change in serum cholesterol that was reported by Grande et al. (79).

Keys et al. (80) used some of the same data previously reported (27), plus new data on 22 schizophrenic men on controlled diets, plus data reported by others to emphasize the individual variability in the serum cholesterol response to diet. Based on analysis of data from 36 men, they found a correlation coefficient of 0.71 between a person's serum cholesterol concentration at the start and the concentration at the end of a cholesterol feeding experiment. They developed an equation to predict an individual's response from his initial serum cholesterol level. By inspection of one table describing individual responses, it appears that about half the subjects responded to dietary cholesterol and half did not.

Further experiments with fat-modified eggs. Brown and Page (81) also tested eggs enriched with polyunsaturated fatty acids by feeding hens sunflower seed. Five normocholesterolemic men, 26 to 33 years, received selected foods supplemented by two eggs, either regular or unsaturated. Both types of eggs, which added about 500 mg of cholesterol per day to the basic diet (210 mg/day), maintained serum cholesterol concentration at the usual level, while the egg-free basic diet lowered serum cholesterol by about 40 mg/dl. They concluded not only that eggs contributed to an elevated serum cholesterol level, but that there was no difference in the effect of unsaturated eggs.

Response to dietary cholesterol independent of type of fat. In a study focused principally

on assessing the effects of type of fat on serum cholesterol concentration, Hegsted et al. (26) also varied the amount of cholesterol contributed by egg yolk. The subjects were 20 institutionalized schizophrenic men, age 38 to 57 years. Men whose serum cholesterol concentrations were less than 200 and more than 300 mg/dl were excluded; their mean level on a house diet was 225 mg/dl. On a control diet containing 331 mg cholesterol per day, the mean serum cholesterol concentration rose to 250 mg/dl and then fell to 220 mg/dl over a 6-month period. Four week test periods were used. The control and test diets were mixtures of low fat foods supplemented by various oils to provide between 22 and 38% of total calories from fat; the total caloric intake, adjusted to maintain constant body weight, ranged from 2200 to 3000 cal/day. Daily cholesterol intakes tested were 116, 300, and about 600 (437 to 686) mg/day. Change in serum cholesterol concentration was measured by comparing the mean level at three and one-half and four weeks of the test diet exposure with the mean values on the control diets immediately preceeding and following the test period.

Using the results obtained with a wide variety of fats and oils, regression equations were computed which related the change in serum cholesterol concentration to the proportions of saturated, monounsaturated, and polyunsaturated fatty acids, and to the amount of cholesterol ingested per day (expressed as 100 mg of cholesterol per day). In the equations which gave the best fits to the observed responses, the coefficients for cholesterol intake ranged from 3 to 9, depending both on the experiment and on the number of variables that were included in the regression equation. An equation based on cholesterol intake alone gave a coefficient of 13, but this equation predicted change in serum cholesterol poorly.

Inspection of the responses to dietary cholesterol intakes of 116, 306, and 686 mg/day, with each intake combined with oils of widely differing degrees of saturation of fatty acids (safflower, olive, and coconut), disclosed a fairly constant linear relationship between dietary cholesterol and serum cholesterol of 5 mg/dl per 100 mg of dietary cholesterol per day. Thus, the response of serum cholesterol was independent of the type of dietary fat when the amount of fat was constant, and there appeared to be no interaction with type of fat.

Effect of dietary cholesterol on cholesterol metabolism. Wilson and Lindsey (82) examined intensively the effects of cholesterol feeding on cholesterol balance in two healthy young men. They did not report the effects of added cholesterol on serum cholesterol concentration, but did compare the effects of egg yolk cholesterol with those of crystalline cholesterol on several indicators of cholesterol metabolism. They detected no differences in metabolic effects between the two sources of cholesterol. Approximately 25 to 40% of serum cholesterol was derived from dietary sources when intake of cholesterol was high from either source.

Dietary cholesterol with saturated fat. Wood et al. (83) studied the mechanism of the effects of dietary fatty acids on cholesterol metabolism in five subjects (one male, four females), 49 to 80 years of age, with "apparently normal lipid metabolism." After a period on a saturated fat diet containing no cholesterol, 750 mg cholesterol per day were added. This produced a "significant but modest increase in plasma-cholesterol," but the figures were not given. Inspection of the graph of one subject indicates that the rise was from about 130 to 170 mg/dl.

Dietary cholesterol effects in obese subjects. Galbraith et al. (84) examined the effects of low fat, low calorie diets on serum lipid levels in 6 very obese adults, 20 to 35 years old, in a metabolic ward. On a 900-cal diet containing 1400 mg/day of cholesterol, the total serum cholesterol fell in all subjects by an average of 54 mg/dl; individual changes ranged from 38 to 77 mg/dl.

Limited response in hospitalized patients. Splitter et al. (85) reported studies with feeding egg yolk to six metabolic ward patients and seven ambulatory healthy volunteers. The six patients were a 61-year-old Filipino male with cerebral vascular disease; a 55-year-old black female with diabetes, obesity, and hypercholesterolemia; a 60-year-old obese white female with diabetes and cardiovascular disease; a 63-year-old American Indian; a 58-year-old diabetic with severe peripheral vascular disease; and a 62-year-old man with osteoporosis. The seven ambulatory normal subjects ranged in age from 25 to 58

years and had initial serum cholesterol levels ranging from 183 to 276 mg/dl. Feeding supplementary egg yolk lipids in amounts which provided up to 5000 mg of cholesterol per day produced elevations in serum cholesterol concentration in only three of the 13 subjects. Crystalline cholesterol dissolved in simulated egg yolk glyceride mixture did not elevate serum cholesterol in two subjects tested. Otherwise, it is difficult to summarize the results of this report.

The Faribault Study. The National Diet-Heart Study (86) was the largest and most thoroughly planned and executed trial of dietary control of serum cholesterol concentration ever conducted. In five American cities, about 1000 free-living men were enlisted in a plan to eat specially prepared foods for 1 year. The foods were selected and prepared to produce three diets as outlined in Table 2.

Seven-day diet records showed that men on test diets 1 and 2 consumed less polyunsaturated fat and less cholesterol than was planned, and that men on the control diet consumed considerably less cholesterol (332 mg/day) than was planned. Under these conditions, each of the two test diets produced a reduction in serum cholesterol concentration averaging about 26 mg/dl below the baseline level of 230 mg/dl. Presumably because the men on the control diet changed their diets, their serum cholesterol concentrations also dropped by about 6 mg/dl. The decrease in the subjects on the two test diets was about 60% of that predicted by regression equations based on previous smaller studies, and the discrepancy was attributed to failures to adhere closely to the prescribed diets. In supplementary studies with institutionalized persons, the decrease in serum cholesterol on the same diets was substantially more, and more closely approached the predicted levels.

Although this study conclusively demonstrated a relationship between diet and serum cholesterol levels, and demonstrated that serum cholesterol could be lowered by dietary changes, the major study in free-living men did not provide any information regarding the relative importance of dietary cholesterol compared to amount or type of fat in controlling serum cholesterol. As part of the project, a supplementary study conducted in a Minnesota mental hospital was designed specifically to determine independent effects and interactions between the two dietary factors (87).

Base diets, low in fat and cholesterol, were designed to be fed with a combination of supplements to give different combinations of types of fat and levels of cholesterol intake. Cholesterol was provided by frozen egg yolks estimated to add about 495 mg of cholesterol per day to the 126 to 154 mg of cholesterol in the base diet. An imitation egg yolk, made of glycerides and protein to simulate egg yolk without its cholesterol, was added to the base diet to make the low cholesterol diet. Four different fat supplements gave unsaturated and saturated fat combinations, each type from two different sources of fats. One pair of fats was used with one group of men, and the other pair with another group. One saturated fat mixture, rich in butter, contained more cholesterol than the unsaturated fat with which it was paired.

Two groups of subjects, one of about 113 men and the other of about 85 men, living in different cottages, consumed each experimental diet for 10 weeks. The resulting serum cholesterol concentrations, based on the means of samples taken at 3 and 10 weeks after the initiation of each diet, are shown in Tables 3 and 4.

In summary, the addition of about 500 mg cholesterol to an unsaturated fat diet resulted in elevations of 4 and 7 mg/dl; to a saturated

TABLE 2
Planned test diets of the National Diet-Heart Study (86)

Component	Diets		
	Test 1	Test 2	Control
Total fat (% total cal)	30	40	40
Saturated fatty acids (% total cal)	<9	<9	≤18
Polyunsaturated fatty acids (% total cal)	≥15	18–20	≤7
Cholesterol (mg/day)	350–450	350–450	650–750

TABLE 3
Mean serum cholesterol concentrations (mg/dl) of 113 men in Hickory Cottage on four test diets (87)

		Type of fat		Difference
		Unsaturated	Saturated	
Cholesterol	Low	180	198	+18
	High	184	211	+27
Difference		+4	+13	

TABLE 4
Mean serum cholesterol concentrations (mg/dl) of 85 men in Springdale Cottage on four test diets (87)

		Type of fat		Difference
		Unsaturated	Saturated	
Cholesterol	Low	190	219	+29
	High	197	233	+36
Difference		+7	+14	

fat diet, it resulted in elevations of 13 and 14 mg/dl. Thus, there appeared to be an interaction between cholesterol and saturated fat in the effect on serum cholesterol concentration. The main effects of both cholesterol and fat were statistically significant in each group of subjects; the interaction was significant in one but not the other. When data from both groups were combined, the interaction was significant. The results from subjects in Springdale Cottage (Table 4) are somewhat confounded because the saturated fat diets contained more cholesterol than did the unsaturated fat diets, and this difference may account for the apparent greater effect of saturated fat in the Springdale group than in the Hickory group.

This experiment conducted as part of the National Diet-Heart Study is the largest and best controlled study addressed directly to the question of independent effects of cholesterol and fat and of their interactions. Each 100 mg cholesterol per 1000 kcal/day added about 2.6 mg/dl to the serum cholesterol concentration in the presence of unsaturated fat, and about 6.7 mg/dl in the presence of saturated fat. From these results one might estimate that the 600 mg/day of cholesterol in the average American diet of 2400 kcal/day contributes about 14 mg/dl to the total serum cholesterol concentration, and that if this intake were reduced to 300 mg/day, the reduction in serum cholesterol would be about 7 mg/dl. If the diet were changed to a highly unsaturated fat diet, the contribution of dietary cholesterol would be about half that. Unfortunately, the report included no data by individual subjects, and gave no information regarding the frequency distribution of responses to dietary cholesterol.

Response of patients with hypercholesterolemia or ischemic heart disease. Grundy and Ahrens (88) studied cholesterol absorption and synthesis in six subjects with ischemic heart disease or hypercholesterolemia or both. In all but one the cholesterol intake was constant; in the other, a normocholesterolemic patient with ischemic heart disease, the plasma cholesterol rose from 180 to 199 mg/dl when cholesterol intake was raised from 34 mg/day to 1587 mg/day. Since cholesterol was not changed in the other subjects, no estimate of their response to dietary cholesterol could be made.

Summary and critique of results of 1960 to 1969. This decade witnessed a shift in the consensus of most investigators regarding the relationship of dietary cholesterol to serum cholesterol concentration, and thereby its probable relationship to atherosclerotic disease. In several well controlled experiments with humans, various levels of dietary cholesterol were fed in combination with different types of fat. These experiments involved substantial numbers of subjects under both metabolic ward conditions (70–72) and institutionalized or free-living persons (26, 27, 69, 78, 79, 87). Dietary cholesterol in formula diets and in natural foods elevated serum cholesterol. Dietary cholesterol given as crystalline cholesterol or as egg yolk elevated serum cholesterol, but egg yolk sometimes was more effective. Some experiments used daily doses of cholesterol far above those ordinarily consumed by humans, but many experiments showed an effect with doses well within the range of intake by persons living in the United States. In experiments designed to test for an interaction with type of fat, the results were equivocal, but the largest study showed a slight interaction.

The experiments by Mendez et al. (76, 77) with cholesterol feeding to Guatemalan Indian children gave conflicting results: feeding eggs led to an increase in serum cholesterol, but feeding pure cholesterol dissolved in oil

led to a decrease. The prior nutrition of these children, quite different from that of urban Guatemalan children or of children in affluent countries, may have affected their response.

In conclusion, it was clear from controlled experiments in humans that cholesterol did influence serum cholesterol concentration, but to a lesser degree than did saturation of dietary fatty acids. However, uncertainty remained regarding the degree of the effect, the shape of the dose-response curve, whether the effect was modified by the type of dietary fat, and whether cholesterol in its crystalline form was absorbed as well as that in natural foods. None of the larger studies indicated the frequency distribution of responses to dietary cholesterol, and therefore it is not possible to estimate the proportion of persons whose serum cholesterol concentrations were appreciably affected by dietary cholesterol.

1970 to 1978

Waning activity in investigation of dietary cholesterol. Interest in dietary cholesterol seemed to decrease after 1970 as emphasis shifted to clinical trials of the effectiveness of hypolipidemic diets and drugs in preventing ischemic heart disease. Despite the repeated demonstrations of an effect of dietary cholesterol on serum cholesterol in the previous decade, controversy continued and additional experiments were conducted to explore its effects under various conditions and in various types of subjects, including hyperlipidemic subjects.

Effects of fat at different cholesterol intakes in hyperlipidemia. Grundy and Ahrens (89) studied 11 hyperlipidemic subjects, of which nine were hypercholesterolemic type II patients and two were hypertriglyceridemic types IV and V patients. Six hypercholesterolemic subjects received low dietary cholesterol formulas (46 to 83 mg/day); three subjects received moderate dietary cholesterol (457 to 679 mg/day); and the two hypertriglyceridemic subjects, received moderate daily intakes of cholesterol (586 to 679 mg/day). These results clearly showed that fatty acid saturation affected total serum cholesterol concentration in these hyperlipidemic subjects, regardless of the dietary cholesterol intake. However, the investigators did not expose the same subjects to different dietary cholesterol intakes, and a comparison of dietary cholesterol effects with fatty acid effect is not possible.

Dietary cholesterol effects in hyperlipidemic subjects. Quintão et al. (90) studied 8 patients for up to 19 weeks under metabolic ward conditions with liquid formula diets containing 40% calories from two vegetable oils. The patients ranged from 14 to 67 years of age and all had either hypercholesterolemia or ischemic heart disease or both. They were fed up to 4,000 mg of cholesterol per day. Since the primary focus of the study was sterol balance rather than effect on plasma cholesterol, they did not attempt to summarize the increment in plasma cholesterol concentration due to supplementary dietary cholesterol. They commented, "What is most impressive in these data is the patient-to-patient variability in response to the imposition of a high cholesterol intake. Plasma cholesterol concentrations rose in five patients, and fell or remained essentially unchanged in three." Inspection of the graphs showing daily plasma cholesterol levels illustrates readily the difficulty one would have in summarizing the response to dietary cholesterol, and most certainly no generalization is possible.

Dose-response to different cholesterol intakes. Mattson et al. (91) fed 56 male prison inmates 21 to 48 years old (median age, 26) emulsified formula diets with nearly identical fatty acid compositions (40% saturated and 12% polyunsaturated) but with egg yolk cholesterol added to provide none, 106, 212, and 317 mg/1000 Kcal per man per day. In an initial 21-day period on cholesterol free diet, the serum cholesterol concentration dropped from about 180 mg/dl on regular prison fare to about 160 mg/dl. The four test diets containing added cholesterol were fed for 42 days and produced elevations in serum cholesterol concentration that equaled 12 mg/dl for each 100 mg of cholesterol added per 1000 kcal. The relationship was linear over the range of dietary cholesterol, the upper limit of which approximated the usual (not the upper limit of) American intake. From this relationship, one would estimate that the usual American adult intake of cholesterol combined with the usual fat intake (40% of calories from fat; P/S ratio about 0.3) contributes about 40 mg/dl to the total serum cholesterol concentration.

High cholesterol reducing diet. Rickman et

al. (92) measured the effect of the Stillman diet on the serum cholesterol levels of 12 healthy men between 21 and 30 years of age. This diet provided 50% of calories from fat, largely saturated fat, and about 1200 mg of cholesterol per day. The average serum cholesterol rose from 215 to 248 mg/dl ($P < 0.001$). Two of the 12 subjects showed no change in serum cholesterol concentration. It was not possible to determine the relative contributions of type of fat and of cholesterol to serum cholesterol changes in this experiment.

Absorption of cholesterol in hypercholesterolemic subjects. Connor and Lin (93) studied the absorption of cholesterol from a single test meal in 15 normocholesterolemic and 6 hypercholesterolemic men. These men were maintained on varied levels of daily cholesterol intake. Absorption of cholesterol averaged about 45% under all conditions in both types of subjects. Radiolabelled crystalline cholesterol was absorbed just as well as cholesterol in egg yolk labeled by feeding hens labeled cholesterol. The results were interpreted to indicate that differences in absorption of cholesterol did not account for human type II hypercholesterolemia; that percentage absorption was not affected by the total amount of cholesterol ingested; and that crystalline cholesterol was absorbed as well as cholesterol naturally occurring in egg yolk. No data on serum cholesterol levels at different levels of dietary intake were presented.

Manipulation of egg consumption in free-living subjects. Slater et al. (94, 95) conducted three experiments with healthy male free-living volunteers. In a prelininary experiment, 15 students 20 to 30 years old consumed two additional eggs at breakfast for 6 weeks and none for the next 4 weeks. All meals were eaten in the medical school cafeteria. The average plasma cholesterol concentration rose to a peak about 10 mg/dl above control initial levels 3 weeks after starting the egg feeding period, and then fell to about 7 mg/dl above the initial level at the end of the test period. In the following control period, it returned to the control level.

In a second experiment, 21 middle aged men 41 to 66 years old (average, 51 years) consumed an average of one extra egg per day for 8 weeks and no eggs for 2 weeks. All meals, including the eggs, were eaten at home. The mean plasma cholesterol concentration increased to 211 mg/dl after 3 weeks on extra eggs, a significant elevation over the 198 mg/dl initial level. However, at the end of the extra-egg eating period, the plasma cholesterol had fallen to 200 mg/dl, obviously not different from the initial level, and nearly identical to the follow-up period in which no eggs were consumed.

The third experiment involved 25 young men, average age 24 years, selected for negative family history of diabetes, cardiovascular disease, or hypertension. They also were selected for an initial low plasma cholesterol concentration (mean, 171 mg/dl). Their usual cholesterol intake was estimated from a three-day diet record to average 343 mg cholesterol/day. During an 8 week test period, each student consumed 2 additional eggs per day, raising his estimated average intake of cholesterol to 793 mg/day. The average plasma cholesterol concentration at all weeks of the egg-eating period were lower than the concentration in the initial period. When each subject was used as his own control, there were no individual elevations in plasma cholesterol concentration.

The authors pointed out that the results of the third experiment were biased due to their selection of subjects. If individuals consuming a usual diet happen also to be responsive to dietary cholesterol, they probably will have higher plasma levels of cholesterol than the average. Therefore, since one of the criteria for selection as a subject was an initial *low* serum cholesterol concentration, the responsive individuals may have been excluded by the selection process. The decline in plasma cholesterol after an initial rise, observed in the first and second experiments, is often seen in both humans and animals placed on cholesterolemia-inducing diets, and presumably represents an adaptation to the diet.

Porter et al. (96) enlisted 114 free-living men from a university faculty between 30 and 60 years old (mean age, 44.6) to eat one whole egg daily or to avoid eating eggs for 3 month periods. The men were divided into two groups and a cross-over design was used to reduce the effects of seasonal trends and to compensate for carry-over effects. A 4-day diet record taken during the middle of each period was used to establish the similarity of the two diets consumed with respect to com-

ponents other than eggs and cholesterol. The added egg per day increased the cholesterol intake by 235 mg/day. The mean serum cholesterol concentrations of both groups were about 225 mg/dl at the start of the experiment, and did not change either after the added-egg period or the no-egg period. The investigators called attention to the "high individual variability of response as shown by analysis of variance and large standard deviations of mean changes." However, no attempts were made to identify the characteristics of the men who responded in comparison with those who did not.

Miscellaneous experiments. McDonald et al. (97) fed 10 healthy young men diets similar except for eggs for 21 days. The variations in diet ranged from no eggs to six eggs per day. Serum cholesterol concentration increased approximately linearly, but the increment was small. With two eggs per day, amount of cholesterol equal to the usual United States intake, the increment was 10 mg/dl; with six eggs per day, it was 29 mg/dl. These changes were ⅓ to ½ the elevations due to egg yolk cholesterol found by Mattson et al. (91). Details of data were limited because the work was published only in abstract form.

Rhomberg and Braunsteiner (98) described a 30-year-old woman who had a serum cholesterol concentration of 940 mg/dl and xanthomatosis. She had been eating 8 to 12 eggs per day, steaks, chickens, and milk as a diet to maintain a low weight. The daily cholesterol intake averaged about 3500 mg/day. After returning to a more usual diet, the serum cholesterol concentration dropped to 188 mg/dl.

Anderson et al. (99) fed 12 young men about 300 mg crystalline cholesterol per day with both saturated and unsaturated fats for 14 days. The cholesterol supplement increased the serum cholesterol concentration by 9 mg/dl with saturated fat and 8 mg/dl with unsaturated fat. In contrast, the saturated fat alone increased serum cholesterol by 36 mg/dl. These results were interpreted to indicate no interaction between dietary cholesterol and type of fat, and independent effects of dietary fat and cholesterol on serum cholesterol concentration.

Nestel and Poyser (100) tested the effects of adding 500 mg of cholesterol daily as egg yolk powder to the diets of nine subjects aged 21 to 64 years who already were consuming about 250 mg/day. Two of the subjects, both males, were normolipidemic; seven, one of whom was female, were hyperlipidemic. Plasma cholesterol concentration after 4 to 6 weeks did not change in five subjects and increased from 26 to 142 mg/dl in four subjects ($P < 0.05$).

In an abstract with only limited details, Mistry et al. (101) reported that cholesterol supplements of 75 to 1500 mg/day given to 14 men produced moderate elevations in serum cholesterol concentration in 10 men fed 750 mg/day and more steeply in four men fed 1500 mg/day.

Quintão et al. (102) studied sterol balance in 27 patients hospitalized for duodenal ulcer or aortofemoral grafts, or maintained in a metabolic ward (these included subjects with hypercholesterolemia and ischemic heart disease). Cholesterol, dissolved in vegetable oils or in capsules, was added to both formula and regular diets. Feeding 10 individuals 3100 to 3400 mg of cholesterol per day increased the serum cholesterol concentration by an average of 52 mg/dl. It is not possible to summarize the response of serum cholesterol to cholesterol intake in the other varied experiments.

Eggs added to diets of hospitalized patients. Kummerow et al. (103) fed 121 hospitalized patients in three cities of the United States and Europe two eggs a day more than the usual convalescent hospital diet. The patients varied widely in cause for hospitalization (some were hyperlipoproteinemic) and age. Both sexes were represented. The data for serum cholesterol levels at intervals up to 54 days after starting the diet supplement were incomplete. The authors emphasized the variability in responses of serum cholesterol to the added cholesterol, pointing out that the serum cholesterol of some patients increased, and that of other patients decreased, while the average values did not change. However, the design of this study, the incompleteness of the data, and the highly biased nature of the subjects prevent making any inferences about the general population.

Summary and critique of results 1970 to 1978. The most informative and convincing experiment of this decade was that conducted by Mattson et al. (91). This experiment tested

the effects of dietary cholesterol at levels of intake from 0 to 317 mg/1000 kcal per day in young institutionalized men. The results showed a linear relationship between dietary cholesterol intake and serum cholesterol concentration within this range of intake, the upper limit of which was about equal to the average United States intake. The accompanying fat was relatively saturated (P/S about 0.3), which may have exaggerated the response slightly if there is an interaction between cholesterol and type of fat.

Several experiments on varying cholesterol intake of hyperlipidemic subjects were reported during this period (89, 90, 93, 100, 102). It is difficult to summarize the effects of dietary cholesterol on serum cholesterol concentration in these experiments, but about half the hyperlipidemic subjects responded to changes in dietary cholesterol with modest parallel changes in serum cholesterol.

Two reports from this period dealt with the practical problem of reducing serum cholesterol in free-living men by adding or removing eggs from the diet (94–96). The results were mixed; some experiments showed a slight but temporary rise in serum cholesterol; others showed no effect. The lack of rigid control of other dietary factors, selection of subjects (in one experiment) to include only those with relatively low serum cholesterol levels, and the modest changes in total cholesterol intake cannot support a firm conclusion that egg yolk cholesterol does not influence serum cholesterol concentration.

Summary of major experimental studies. Table 5 summarizes in condensed form the major experimental studies testing the independent effects of dietary cholesterol on serum cholesterol concentration under rigorously controlled conditions.

Mechanisms other than by influencing serum cholesterol concentration

Possibility of other mechanisms

Practically all observational and experimental investigations of the relationship of dietary cholesterol to atherogenesis have assumed that the effect, if any, was mediated by serum cholesterol concentration. This assumption seemed reasonable in view of the clearly established relationship of serum cholesterol level to risk of atherosclerotic disease, and the association of elevated serum cholesterol to atherosclerosis in experimental animals. The possibility remains that dietary cholesterol could affect atherogenesis without changing the serum cholesterol concentration, which is controlled by several complex interdigitating systems involved in sterol metabolism. Some of the mechanisms that have been suggested are outlined below.

Alteration in lipoprotein profile

Most of the major experiments concerned with dietary cholesterol effects were performed before the distinctive roles of the different lipoproteins were fully appreciated. Therefore, investigators were concerned primarily with the response of total serum cholesterol concentration rather than that of specific lipoproteins. More recently, a few reports have described the association of dietary cholesterol intake with LDL (β-lipoprotein) and HDL (α-lipoprotein) (e.g., Frank et al. (43). More of these are certain to come in the near future, and they may help to resolve the problem.

Alteration in structure or composition of serum lipoproteins

Rudel et al. (104) found that monkeys (*Macaca fascicularis)* fed high cholesterol, high fat diets developed LDL particles that were both larger and heavier than normal. The presence of these particles was associated with more extensive atherosclerosis. Mahley and Holcombe (105) reported that feeding cholesterol to rats caused an increase in very low-density lipoproteins, intermediate density lipoprotein, LDL, and β-very low-density lipoproteins; the appearance of another lipoprotein, HDL_c, in the plasma; and a decrease in the usual HDL. More recently, Mahley et al. (106) reported that addition of four to six eggs per day to the diets of 11 free-living men and women increased an HDL subfraction with greatly increased binding activity for the cell-surface receptors of fibroblasts. The increased activity of HDL appeared regardless of the effect of the eggs on plasma cholesterol concentration. The mean increase in plasma cholesterol during egg feeding over baseline was 11 mg/dl; four of the subjects had an *increase* greater than 10 mg/dl, and two had a *decrease* greater than 10 mg/dl.

Increased turnover rate of lipoproteins

Zilversmit (107) has suggested, on the basis of a variety of experiments in rabbits, that the remnant particles produced when chylomicrons are degraded by lipoprotein lipase are transported across the endothelium and enhance atherogenesis. In this concept, cholesterol absorbed from the intestine and transported in chylomicrons would be transferred directly into the arterial wall from the circulation. Since this process principally occurs in the postprandial period, and since most serum cholesterol and lipoprotein determinations are made in the fasting state, increased dietary cholesterol might augment atherogenesis without affecting the serum or LDL cholesterol concentrations measured in the conventional manner.

Stimulation of mitotic activity of smooth muscle cells

A cholesterol-enriched diet increased the mitotic activity of both endothelial cells and smooth muscle cells of the aortas of swine after only three days of feeding (108, 109). This change was detected along before serum cholesterol was elevated. The components of serum responsible for mediating this effect were not identified.

Other possible mechanisms

Grundy (110) reviewed the many effects of dietary fats and sterols on lipid metabolism. It seems obvious that any increase in absorbed cholesterol will affect some part of the homeostatic system, regardless of whether serum cholesterol concentration is elevated. Either more cholesterol will be stored in the tissue pools, or more will be excreted via the bile acids, or both.

Armstrong et al. (111) fed rhesus monkeys sufficient amounts of cholesterol to raise the serum cholesterol levels of those with intrinsically low concentrations to the levels of those with intrinsically high concentrations. The animals whose serum cholesterol concentrations were raised within the "normal" range showed more extensive intimal thickening than those whose serum cholesterol concentrations were already high.

Otherwise, there is essentially no information on the issue of whether a more rapid turnover of cholesterol in the body, serum cholesterol concentration remaining constant, will result in more or less atherosclerosis. For practical purposes, this question can be answered only in experimental animals.

Summary and critique of other potential mechanisms

The possibilities that have been suggested by observations in experimental animals can be considered only as highly speculative for the present. All of the cholesterol feeding experiments in animals involve the concurrent feeding of fat, and in none of the above experiments was there a control group for the fat alone (that is, fat without cholesterol). The observation that egg supplementation alters the lipoprotein profile and in particular alters the properties of HDL independently of serum cholesterol concentration offers a new potential mechanism that needs further exploration and confirmation.

Exceptions

The Masai of East Africa

Initial description. In 1964, Mann et al. (112) conducted a field survey of cardiovascular disease among the Masai of East Africa. This tribe of nomadic cattle herders was reported to consume predominantly milk, meat, and occasionally blood, and presumably had a high intake of animal fat and cholesterol. They called attention to the apparent discrepancy in 400 men, women, and children examined between the high fat, high protein, and high cholesterol diet on the one hand and the low serum cholesterol concentrations, averaging 125 mg/dl, and near absence of atherosclerotic disease on the other hand.

Dietary and metabolic studies. Ho et al. (113) verified that the milk of Masai cattle was high in butterfat and that it contained about the same amount of cholesterol as American milk. They estimated that, during each day of the wet season, the average Masai adult consumed about 3000 kcal, 66% of which was derived from fat, and about 600 mg of cholesterol. During the dry season, they estimated that the Masai would have a higher intake of cholesterol and fat. Although the investigators examined the composition of the milk thoroughly, they did not measure directly the actual food consumption of Masai during any season.

TABLE 5
Summary of selected experimental observations in humans on the effects of dietary cholesterol on serum cholesterol concentration

Reference	Subjects		Diet					Serum cholesterol concentration			Comment
	Description	Age	Basal diet and % cal from fat	Cholesterol source	Length of periods	No. of subjects	Cholesterol	Mean	Mean elevation attributed to dietary cholesterol	Mean elevation per 100 mg/1000 kcal	
		yr			*days*		*mg/1000 kcal*		*mg/dl*		
Beveridge et al. 1960 (69)	53 male, 14 female free-living university students		Formula, 30% fat, butter oil	Crystalline	8	9	0	171			
						6	26	173	2	8	
						9	53	175	4	8	
						9	105	177	6	6	
						9	211	188	17	8	
						6	421	194	23	5	
						10	842	217	46	5	
						9	1684	204	34	2	
Connor et al. 1961 (70)	Male prison volunteers (1 hypercholesterolemic, 2 overweight)	40–45	Formula, 40% fat, peanut oil	Egg yolk	23	1	567	316	94	17	Metabolic ward
					23	1	948	273	115	12	
					23	1	1926	253	98	5	
					23	3	892	213	61	7	
Connor et al. 1961 (71)	Male prison volunteers	41–52	Formula, 40% fat, mixed oils	Egg yolk	21	2	158	245	66	42	Metabolic ward
						2	317	277	64	20	
						2	475	261	77	16	
				Crystalline	21	2	400	219	16	4	
						2	800	235	19	2	
						2	1200	222	24	2	
Steiner et al. 1962 (74)	3 male, 2 female hospital patients	50–63	Formula, 45% fat, olive oil	Crystalline	56	5	0	211			
							1500	292	81	5	
Erickson et al. 1964 (78)	Male prison volunteers	29–45	Formula, 41% fat, mixed oils	Egg yolk	35	42	0	191			7 treatment, 4 replication, incomplete Latin square design
							306	216	25	8	
Grande et al. 1965 (79)	Male, physically healthy confined schizophrenics	35–65	Mixed food, 45% fat	Crystalline	14–21	22	18	196			Cross-over design
							559	223	27	5	
				Egg yolk	21		144	212	16	11	

Hegsted et al. 1965 (26)	Male, physically healthy confined schizophrenics; serum cholesterol 200–300 mg/dl	38–57	Mixed food, 38% fat	Egg yolk	28						No interaction with type of fat
			Safflower oil			10	39–53	169			
							102–139	182	13	7	
							229–312	198	29	11	
			Olive oil			10	39–53	221			
							102–139	234	−23	−6	
							229–312	248	27	10	
			Coconut oil			10	39–53	266			
						20	102–139	287	3	3	
						10	229–312	287	21	4	
National Diet-Heart Study, Faribault Second Study 1968 (87)	Male, confined mental patients	40+	Mixed food, 36–42% fat	Egg yolk	70						Factorial design
	Hickory Cottage		P/S 2.3			81	43	180			
			0.5				148	184	4	3	
							43	198			
							149	211	13	9	
	Springdale Cottage		P/S 1.0			108	66	190			
			0.1				195	197	7	4	
							110	219			
							217	233	14	9	
Mattson et al. 1972 (91)	Male prison volunteers	21–48	Formula, 40% fat, mixed oils	Egg yolk	42	10	0	165			
						11	106	175	13	12	
						13	212	181	24	11	
						14	317	198	42	13	

The average serum cholesterol concentration of 254 Masai over 15 years of age was 135 mg/dl. The season of the year during which samples were taken was not stated. There was no significant increase of serum cholesterol with age. Serum lipoproteins separated by paper electrophoresis showed β-lipoprotein lower than, and α-lipoprotein about equal to, those of American persons of similar age and sex.

The investigators also verified the lack of severe atherosclerosis in the Masai by examining aortas and coronary arteries from nine men and one woman from 22 to 67 years of age. The aortas contained a few fatty streaks and fibrous plaques but no complicated lesions. Coronary arteries likewise were only slightly involved with atherosclerosis. A similar extent and severity of atherosclerosis were found in hearts and aortas of 50 autopsied Masai by Mann et al. (114).

To test the Masai's response to dietary cholesterol, Ho et al. (113) fed 23 young men in a boarding school a prepared formula diet of 3605 kcal/day for 8 weeks. Of the calories 34% were derived from fat provided by a nondairy coffee creamer. To the diets of 11 men 20 to 24 years old they added 2000 mg of cholesterol per day; and to the diets of 12 men 19 to 22 years old they added no cholesterol. Both groups gained weight. Those fed cholesterol absorbed, on the average, about 33% of the ingested cholesterol. The average serum cholesterol concentrations of both groups rose from about 125 mg/dl before the trial to about 170 mg/dl during the test period, but the average serum cholesterol concentration of the cholesterol-fed group remained identical to that of the control group. The cholesterol-fed Masai showed marked suppression of endogenous synthesis. The authors interpreted these findings to indicate that the Masai had adapted physiologically to their unusual diet, and that they possessed a genetic resistance to the cholesterolemic effects of dietary cholesterol. The results of this study are summarized in another report by Biss et al. (115).

Day et al. (116) compared serum cholesterol levels of 27 rural Masai with those of 26 Masai who had been living in cities for 10 or more years. The urban Masai had an average serum cholesterol level of 203 mg/dl; the rural Masai, 160 mg/dl. The investigators believed that the urban Masai did not consume more fat or cholesterol than did the rural Masai, but they did not perform detailed dietary surveys. They concluded that the low serum cholesterol of rural Masai was not genetic, but was determined by environmental factors other than diet.

Critique of studies of the Masai. Clearly, Masai in their natural habitat have low serum cholesterol concentrations, little or no advanced atherosclerosis, and practically no atherosclerotic disease. One test of dietary cholesterol for 8 weeks in 23 young men (11 experimental, 12 control) showed that added cholesterol was absorbed, but the serum cholesterol rose to about the same amount (35 mg/dl) in both groups. No direct measurements of the actual dietary intake of free-living Masai have been reported. Urban Masai show serum cholesterol levels about equal to those of Europeans (and of United States residents), and therefore are not totally immune to hypercholesterolemia. The Masai are quite different from other African tribes in skin color and body build, and it is conceivable that as a result of evolutionary adaptation or of genetic drift in an isolated population, a large proportion of individuals may possess resistance to dietary cholesterol, as do some individuals from any population. We conclude that more accurate measurement of dietary intake and confirmation of their resistance to dietary cholesterol are necessary before they are cited as showing unusual resistance to dietary cholesterol intake. The association of a low frequency of atherosclerotic disease with low serum cholesterol concentrations conforms to findings elsewhere.

The Arctic Eskimo

Estimates of atherosclerosis and atherosclerotic disease. Eskimos, who subsist on a diet rich in meat, have been cited by many investigators as having a low incidence of atherosclerotic disease and are sometimes cited as exceptions to the association between animal fat and atherosclerosis. The low frequency of atherosclerotic disease observed may be due to the small proportion of persons living to more than 40 years of age and to difficulties in ascertaining accurately the causes of death under Arctic conditions. Arthaud (117) tabulated the causes of death

among 339 autopsied Alaskan natives, including both Eskimos and Indians. Among 18 autopsies of Eskimos 51 to 60 years of age, four were attributed to cardiovascular disease, type not specified. No estimates of atherosclerosis in the other cases were given. Among 35 adults dying of cardiovascular disease, four had cerebral vascular disease, four had myocardial infarction, two had abdominal aortic aneurysms, two were designated "generalized atherosclerotic cardiovascular disease," and one had mesenteric arterial thrombosis. Sudden deaths were not likely to be autopsied. Thus, although the many other reports describing the apparent paucity of atherosclerotic disease may be true for the total population, clearly atherosclerosis does occur with appreciable frequency and severity in the middle-aged Eskimo.

Bang et al. (118) found the average serum cholesterol concentration of 61 Eskimo men over 31 years to be 233 mg/dl; of 69 women over 31 years, 222 mg/dl. These levels were lower than those of Danes living in Denmark, and also were lower than those of 25 Eskimo women living in Denmark. They did not measure dietary intakes, but cited numerous other observations of the high meat intake of Eskimos.

Feldman et al. (119) measured the cholesterol intake of eight Alaskan Eskimos 39 to 66 years of age for 3 weeks. Cholesterol intake ranged from 420 to 1620 mg/day, with an average caloric intake of about 3000 kcal. About 50% of calories were derived from fat. The investigators did not examine the relationship between cholesterol intake and serum cholesterol concentration in these subjects, but they did measure cholesterol absorption, which was about 50%. The mean total serum cholesterol concentration of 168 subjects more than 8 years of age was 221 mg/dl, and of those 60 to 82 years, 250 mg/dl. Young Eskimos living in a boarding school on an American type diet ingested an estimated 380 mg cholesterol per day, and their serum cholesterol levels were 137 mg/dl. The authors point out that during other times of the year, the Eskimos living under their usual conditions would have a lower cholesterol intake. They cited the numerous reports that atherosclerotic disease is rare among Eskimos, but made no direct observations on this point. They concluded that the "data suggest a direct response of plasma cholesterol to dietary cholesterol."

Feldman et al. (120) compared two groups of Alaskan Arctic Eskimos, one group living on a typical Eskimo diet high in fat and the other living on a typical Western institutionalized diet. Those on the Eskimo diet (76 males, 88 females, 6 to 82 years of age) ingested about 900 mg of cholesterol per day and had an average serum cholesterol concentration of 221 mg/dl; those on the Western diet (31 adolescents) ingested about 380 mg of cholesterol per day and had an average serum cholesterol concentration of 144 mg/dl. Other differences in the fat and carbohydrate contents of the diets prevent attributing the difference to dietary cholesterol alone.

Critique of studies of Eskimos. The available evidence indicates that atherosclerotic disease may be infrequent in the Arctic Eskimo, but the low frequency probably is due to their high mortality rate before they reach middle age. Atherosclerotic disease occurs frequently in middle aged and elderly Eskimos. During some parts of the year, they ingest large quantities of cholesterol, but their serum cholesterol concentrations also are high. A direct relationship of serum cholesterol concentration to dietary cholesterol is difficult to establish because of the confounding by other dietary variables. However, the Eskimo does *not* appear to be an exception to the usual association between dietary cholesterol, hypercholesterolemia, and atherosclerotic disease.

The Indians of the Southwestern United States

Navajo Indians. The Navajo Indians also are cited as exceptions to the association between a high fat, high cholesterol diet, high serum cholesterol concentrations, and atherosclerotic disease. The low incidence and prevalence of atherosclerotic disease have been described (121–124). The serum cholesterol concentrations usually were slightly lower than the United States average, smoking was of low intensity, and blood pressures were low. The diet was described as similar to that of the average American diet, with much animal fat as stewed mutton. However, no quantitative data on dietary intake were presented in these reports, and no measurements of dietary cholesterol were made. Consequently, we can draw no conclusions from

the Navajo Indians regarding the relationship of dietary cholesterol to serum cholesterol concentration or to atherosclerotic disease.

Pima Indians. The Pima Indians, who have an unusually high prevalence of diabetes mellitus and gallbladder disease, also have been studied as possible exceptions. Reid et al. (125) found a mean daily cholesterol intake of 489 mg in 277 Pima Indians. The mean serum cholesterol concentration of this group was 179 mg/dl. Five years later, Savage et al. (126) found the mean serum cholesterol concentration of adult Pima Indians to be about 190 mg/dl. With a lower cholesterol intake than the average American adult, and a fat intake about equal to that of the average American adult, the lower average serum cholesterol concentration of the Pima Indian may be due in part to diet and also to other unidentified factors. Available evidence does not indicate that Pima Indians are an exception to the relationship between dietary cholesterol and serum cholesterol found elsewhere.

Reviews of the dietary cholesterol-atherosclerosis issue

Most reviews of the diet-heart problem deal with the manipulation of serum cholesterol concentration by alteration of more than one dietary component, and the resulting effect (or lack of effect) on serum cholesterol level, atherosclerosis, and clinical disease manifestations. The reviews that confront directly the issue of dietary cholesterol as distinct from dietary fat, and particularly as distinct from type of dietary fat, are surprisingly few. None, provides a comprehensive and critical review of the question of whether dietary cholesterol has an independent effect on serum cholesterol concentration, atherosclerosis, or atherosclerotic heart disease.

Keys (127) reflected the prevalent opinion of the 1950's when, in reviewing the contribution of diet to coronary heart disease, he contrasted the sensitivity of several animal species to dietary cholesterol with the high resistance of the human. In concluding a brief review of this one aspect of the diet-coronary heart disease issue, he stated that "for all practical purposes the dietary cholesterol variable may be disregarded. . . . "

Ahrens et al. (63) in the same year reviewed in detail the experiences of the Rockefeller Institute group with the serum cholesterol-lowering effects of polyunsaturated fats. In an extensive review of nutritional effects on serum lipid concentrations, Ahrens again (128) concluded that "serum cholesterol levels *ordinarily* are independent of dietary cholesterol intake in man."

Katz et al. (129) reviewed the knowledge about the relationship between nutrition and atherosclerosis in both man and experimental animals. Most of the review deals with observations on saturation of fatty acids. The specific contribution of dietary cholesterol alone received only slight mention, and no definite conclusion was stated. They commented that both cholesterol and saturated fat alone were relatively ineffective in inducing hypercholesterolemia in experimental animals, but that the combination of the two was particularly effective.

Connor (18) reviewed the role of cholesterol as an independent contributor to elevation of serum cholesterol concentration and to coronary heart disease. He cited the work of his group and that of Beveridge et al. (67, 69) as the principal experimental human evidence, and described the evidence from experimental animals. He also compared the average daily cholesterol intake per person for 24 countries with the death rates for arteriosclerotic and degenerative heart disease (see previous section, National or Regional Population Studies). He recommended that the ideal diet for prevention of coronary heart disease was low in fat, low in saturated fat, and should contain no more than 100 mg of cholesterol per day.

Sprague (130) reviewed the relationship of the commonly suspected environmental agents, including diet, to coronary heart disease, but the dietary factors were not analyzed in detail and he made no distinction between amount of fat, type of fat, and cholesterol.

Wilson et al. (131) reviewed the effects of dietary cholesterol on cholesterol metabolism. They accepted the elevation of serum cholesterol concentration by dietary cholesterol as established, and cited two subjects in which 2280 to 2780 mg of cholesterol per day, either as crystalline cholesterol or as egg yolk, raised the serum cholesterol concentration by 50 to 55 mg/dl. Their inquiry was directed at the question of why the human response was

so limited. They concluded that there were two principal reasons: first, the human has a remarkable ability to resist the absorption of dietary cholesterol, and the two individuals described above had absorbed only 10 to 15% of the cholesterol ingested; and second, hepatic cholesterol synthesis is suppressed in the presence of appreciable intakes of dietary cholesterol.

Dietschy and Wilson (132) reviewed thoroughly the status of knowledge about regulation of cholesterol metabolism. They described the several interdigitating systems that maintain a remarkably constant concentration of cholesterol in the body of man and a number of other species, despite great variations in dietary intake. It is apparent that variations in intake of exogenous cholesterol may affect some of these systems profoundly with only minimal or no effect on the serum cholesterol concentration. They did not discuss the evidence regarding whether dietary cholesterol elevates serum cholesterol.

Connor (133) reviewed the effects of dietary sterols and unsaturated fat on sterol metabolism. On the basis of both published and unpublished data available at that time, he concluded that dietary cholesterol in amounts up to 110 mg/day did not affect serum cholesterol concentration. Dietary cholesterol above 110 mg/day and up to about 600 mg/day increased serum cholesterol. Beyond 300 mg/day, the rate of increase was small with increasing cholesterol intake, an observation suggesting a plateau effect. He also concluded that dietary cholesterol increased the body pool of cholesterol (both plasma and tissue) and increased bile acid excretion, but did not suppress biosynthesis of cholesterol.

Bortz (134) reviewed the factors affecting the concentration of serum cholesterol. He included dietary cholesterol as a significant contributor, but stated that its role was less important than that of dietary fat. He cited nine references bearing on this issue, of which five were concerned primarily with cholesterol absorption and inhibition of endogenous synthesis. This review emphasized the many diverse genetic and environmental factors that influence serum cholesterol, a situation that explains in part why it has been so difficult to find associations between cholesterol intake and serum cholesterol in free-living populations.

Keys (135) reviewed much of the history of developing knowledge about atherosclerotic disease and examined particularly some of the controversial risk factors, such as stress, behavioral pattern, and physical activity. He also reviewed the evidence from exotic peoples that often is cited as contrary to the prevailing opinion on the relationship to serum cholesterol concentration and atherosclerosis—the Arctic Eskimo, the Masai of East Africa, and the American Indian. He concluded that the Masai may be genetically different from most other humans in their cholesterol metabolism, but that the others presented no convincing evidence negating the association. Keys did not mention the question of an independent effect of dietary cholesterol, and did not discuss the relative importance of dietary cholesterol and saturated fat.

Oliver (136) addressed directly the problem of the relationship of dietary cholesterol to serum cholesterol and to coronary heart disease. He concluded (three references cited) that dietary cholesterol made only a minor contribution to plasma cholesterol and therefore was "not an important cause of coronary heart disease, and a moderate regular intake is not harmful." Oliver emphasized and illustrated the many factors other than dietary cholesterol, both environmental and endogenous, that influenced plasma cholesterol concentration. He also emphasized the many factors other than plasma cholesterol concentration (the other risk factors, arterial metabolism, lipoproteins, and others) that influence the eventual outcome of clinically manifest coronary heart disease. Oliver concluded that one major controllable environmental factor among the environmental causes is excessive intake of saturated fat.

Two reviews (137, 138) presented contrasting interpretations of the results published before 1973 on the topic of dietary lipid effects on serum cholesterol concentration. Reiser (137) contended that the saturated fatty acid effect on serum cholesterol was not established, and that most of the dietary control of serum cholesterol was explained by dietary cholesterol intake. His review did not consider many of the reports of original data on the topic, and in particular failed to include one of the largest and most thoroughly conducted studies, that performed in connec-

tion with the National Diet-Heart Study (87). His conclusions ran counter to the then-prevailing consensus that the hypercholesterolemic effect of saturated fats was well established. Reiser's evaluation of the role of dietary cholesterol also estimated a considerably greater contribution of dietary cholesterol to serum cholesterol than generally was believed.

Keys et al. (138) took issue with one after another of Reiser's conclusions, and cited additional reports which Reiser omitted. Curiously, they also failed to cite the National Diet-Heart Faribault Study on the independent effects of dietary cholesterol, although they did discuss the contribution of dietary cholestrol reduction to serum cholesterol reduction in the main study. They concluded that the difference between the two test diets and the control diet of 15 mg of cholesterol per 1000 kcal contributed only 9% of the observed reduction in serum cholesterol concentration related to the two test diets. In summary, Keys et al. (138) estimated that one egg per day added to the 600 to 700 mg of cholesterol consumed by the average physically active American male would increase his dietary cholesterol intake by nearly 50% but would increase his serum cholesterol concentration by only 5 to 6 mg/dl. They did not estimate what effect a *reduction* in cholesterol intake by a similar amount (that is, the amount already contributed by eggs) would have. If the relationship is linear, as found by Mattson et al. (91) the reduction would lower serum cholesterol by the same amount that an addition would increase it; if the relationship is curvilinear, as indicated by previous reports of Keys et al. (27), the reduction would lower serum cholesterol by an amount greater than an addition would increase it.

Truswell (139) stated, among "central facts" with regard to diet and ischemic heart disease, that "plasma cholesterol can be lowered, in most people with hypercholesterolemia and in probably all with average cholesterol concentrations, by reducing the saturated fat and cholesterol of the diet." He went on to state that a major effect is produced by dietary fat, and that the effect of cholesterol " . . . is on the whole smaller, there appears to be more individual variation and it is nonlinear, with the first 300 mg in the diet having the most effect." He commented that most of the expert committees considering dietary recommendations had recommended reducing dietary cholesterol to 300 mg/day, but that several had not made such a recommendation. He concluded that, at least in Britain, the effect of reducing dietary cholesterol by halving egg intake (from about four or five to about two per person per week) would be so small that this recommendation was not advisable.

Truswell (140) reviewed a wide variety of foods and nutrients with regard to their effects on plasma lipids. He accepted the evidence that plasma total cholesterol and LDL cholesterol concentrations were closely (and probably causally) associated with coronary heart disease and related diseases, and that plasma HDL levels were associated with reduced risk. He also accepted the evidence that type of fatty acid affects plasma cholesterol concentrations. His review of dietary sterols concluded that dietary cholesterol (and particularly that in eggs) has little, if any, effect on plasma cholesterol concentration. He cited only seven original investigations, one of which was an abstract and another of which was an anecdotal case report. None of the larger studies of the effects of egg or cholesterol supplementation was cited. Truswell emphasized the individuality of the response to dietary items in a population, and implied that this individuality should be taken into account in making dietary recommendations.

Glueck and Connor (141) reviewed the entire problem of diet and heart disease relationships, ranging from experiments with animals and humans in institutions or metabolic wards to epidemiologic surveys and controlled clinical trials in humans. In their discussion of dietary factors controlling serum cholesterol concentrations, they emphasized the role of dietary cholesterol, perhaps because of the controversy surrounding it, but did not offer an estimate of its importance relative to dietary saturated fat. In reconciling this view with the reported experiments in which one or two added eggs per day did not significantly increase serum cholesterol concentration, they pointed out that several studies had shown that increments producing daily intakes over 500 to 600 mg/day usually had not further increased serum cholesterol concentrations (the plateau effect). They also

suggested that genetically controlled variations in response to dietary cholesterol complicated the detection of a direct relationship in many of the studies reviewed.

Reiser (142) not only deprecated the contribution of dietary cholesterol and fat to serum cholesterol concentration, but also deprecated the role of serum cholesterol itself by contending that levels up to about 250 mg/dl were "normal" and did not confer increased risk of atherosclerotic disease. He supported the concept that hyperlipidemia was a "disease" that should be treated by individual prescription. He also emphasized the variations in response to all dietary components, including cholesterol, in any given population.

Grundy (110) reviewed the effects of dietary fats and sterols on cholesterol metabolism. Citing nine references, he summarized the effect of dietary cholesterol in humans as "only a mild increase in plasma cholesterol in the range of 5 to 10%, and in contrast to animals, marked hypercholesterolemia rarely if ever develops." He also stated that " . . . the animal responses are quantitatively and possibly qualitatively different from those of most human subjects." With regard to lipoprotein changes, he concluded that there was "no evidence that dietary cholesterol causes any changes in the inherent structure or composition of lipoproteins when fed to human subjects," in contrast to evidence from animal experiments. Grundy also suggested the possibility that dietary cholesterol may augment atherogenesis without raising plasma cholesterol concentration. Possible mechanisms could be the transport of dietary cholesterol in chylomicrons directly to the arterial wall, or the formation of abnormal LDL. In conclusion, Grundy stated, "There is renewed interest in the role of dietary cholesterol . . . because recent studies indicate a greater influence [on serum cholesterol concentration] than was previously thought."

Mahley (143) reviewed the effects of both dietary cholesterol and saturated fat on experimental atherosclerosis, with particular emphasis on cholesterol fed to swine, dogs, and patas monkeys; and cited some of the reports dealing with dietary cholesterol and saturated fats. He also reviewed the observations on the changes in lipoproteins, especially HDL, produced by cholesterol feeding in animals and humans. Mahley pointed out that most animal species are more sensitive to dietary cholesterol than is man, and, in contrast, that most animals are *less* senstivie to saturated fat than is man. He summarized the evidence suggesting that dietary cholesterol may affect atherogenesis without elevating serum cholesterol concentration (106, 111), but concluded that this possible relationship had not been definitely established.

Summary and conclusions

Observational epidemiology

Dietary cholesterol is closely associated with both serum cholesterol concentrations and mortality rates for arteriosclerotic heart disease among both large and small population groups. However, the correlation coefficients are reduced to zero or to very low values when associated variables are taken into account. Therefore, simple correlation coefficients cannot be cited as proof of an independent effect of dietary cholesterol, but only as suggestive evidence of a causal relationship.

Among individuals within groups, numerous cross-sectional studies have failed to find a significant independent association of dietary cholesterol with either serum cholesterol concentration or with risk of arteriosclerotic heart disease. The failure to find such correlations, however, does not negate a causal relationship because measurement errors are large and because between subject variability in serum cholesterol concentrations is large regardless of diet.

Controlled experiments with humans

Numerous controlled experiments have demonstrated that the average serum cholesterol concentration of adults increases with increasing dietary cholesterol when the total intake is in the range of 0 to 600 mg/day. Estimates of the average increase in serum cholesterol concentration range from 3 to 12 mg/dl per 100 mg of cholesterol per 1000 kcal. Dietary cholesterol above about 600 mg/day produces no additional effect in most persons. The response tends to be greater if dietary cholesterol is combined with saturated fat.

Fewer hyperlipidemic subjects have been studied, but available data indicate that their

responses to dietary cholesterol are more variable. No estimate of an average response for persons with any specific type of hyperlipidemia can be made.

Frequency distribution of responses to dietary cholesterol

Many investigators and reviewers have commented on the marked variability among individuals in response to dietary cholesterol. Investigators have been concerned primarily with the average effects of dietary manipulations and have presented only mean values, measures of variance, and the results of tests of statistical significance. In no instance in which a substantial number of subjects was tested were the individual base-line levels and the corresponding response levels presented, and no frequency distributions of the responses or the total levels attained presented. Therefore, it is not possible to derive from published data any impression of the relative proportions of those who responded to dietary cholesterol slightly or not at all, and those who responded with marked elevations.

This frequency distribution of responses may be as important in evaluating the impact of dietary changes on a population as the average response, and perhaps more so. It also may be important in determining whether a given dietary change should be recommended for the entire population, or whether it should be recommended only for highly susceptible individuals.

If an average change in serum cholesterol of, say, 10 mg/dl, is made up of a few persons who respond dramatically, and a large number who respond slightly or not at all, the strategy to identify the extreme responders and recommend dietary modification only for them might be advisable. On the other hand, if there is a normal distribution of the individual responses with a small range, separation into the susceptible (or high responders) and nonsusceptible (low responders) would not be feasible and any dietary modification would be recommended for the entire group. In this latter example, the strength of the recommendation would be determined by an evaluation of potential benefit to be gained in reduction of risk for the overall group, based on the mean value.

Factors controlling response

Genetic control probably is a major factor in determining response of an individual to dietary cholesterol. The type of dietary fat (saturated or unsaturated) also may affect the response. Provided there is some fat in the diet (minimal amount not determined), the response to pure cholesterol is, on the average, equal to that in natural foods.

Mechanisms other than via serum cholesterol concentrations

Dietary cholesterol *may* influence atherogenesis even though it does not alter the serum cholesterol concentration. Some evidence indicates that it alters the structure and composition of lipoproteins, and that it stimulates mitotic activity of arterial smooth muscle cells. Other hypothetical mechanisms have been suggested. All such possibilities are speculative for the present, but they deserve further exploration. In particular, we need better information on the effect of dietary cholesterol on specific lipoprotein fractions.

Changing serum cholesterol concentration by manipulating natural foods

The evidence indicates that serum cholesterol concentration can be reduced by the elimination of eggs, the major source of dietary cholesterol, from the diet of free-living adults. The potential for influencing serum cholesterol levels by manipulation of this one major source of dietary cholesterol appears limited, however, because there are many low responders, much cholesterol is derived from other sources, and other dietary factors (such as saturation of fat) affect serum cholesterol concentration. Therefore, it appears that any substantial reduction in serum cholesterol concentration in populations (as contrasted with selected individuals) must be accomplished by changes in a variety of dietary components.

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2. THE RELATIONSHIP OF DIETARY CHOLESTEROL TO SERUM AND LIPOPROTEIN CHOLESTEROL CONCENTRATION AND TO ATHEROSCLEROSIS IN MAN - A REVIEW OF THE LITERATURE: 1979-1986

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I. Introduction

The American Society for Clinical Nutrition in 1978 established a committee to examine the scientific evidence relating selected dietary factors to some of the major chronic diseases afflicting the U.S. population. One of the dietary components considered was cholesterol, which has been implicated as a probable cause of atherosclerosis and its sequelae, coronary heart disease, peripheral vascular disease, and stroke. As part of the committee report, a comprehensive review of each dietary component was prepared, including one of dietary cholesterol (McGill, 1979). The review was limited to dietary cholesterol in humans, and did not encompass the evidence based on animal experimentation. All reports that provided some evidence regarding the effects of dietary cholesterol on serum lipids, serum lipoproteins, atherosclerosis, or one of the clinical manifestations of atherosclerosis, up to early 1979 were examined and summarized. The relationship of serum lipid and lipoprotein concentrations to atherosclerotic diseases was not addressed; this relationship had been adequately reviewed elsewhere, and it was assumed that the evidence for that relationship was firmly established. The conclusions at that time are outlined as follows:

(1) Observational epidemiologic studies have found strong associations between cholesterol intake and serum lipids, atherosclerosis, and atherosclerotic disease on the basis of ecological correlations among populations. However, the associations between dietary cholesterol intake and the same physiologic variables or disease endpoints on the basis of individual correlations among persons within populations have been absent or so weak that they appear to be biologically insignificant.

(2) Controlled experiments in humans have shown that each 100 mg per Kcal of dietary cholesterol, elevates serum cholesterol between 3 and 12 mg/dl as long as the total intake is less than 600 mg/day. Several equations have been developed which predict the average serum cholesterol response to changes in dietary cholesterol.

(3) There is considerable variability among individuals in the response of serum cholesterol to dietary cholesterol. Many persons respond not at all, but a few respond with large increases in serum cholesterol concentration. The frequency distribution of responders is not known.

(4) Dietary cholesterol may alter serum lipoproteins in some ways other than increasing their serum concentrations, but this interesting speculation has very little evidence to support it.

(5) Serum cholesterol can be reduced slightly by eliminating or reducing the consumption of eggs, the major source of dietary cholesterol. Substantial reduction can be expected only in the persons who are highly susceptible to dietary cholesterol.

The present review updates the 1979 review by examining all reports that have appeared since early 1979 which present some evidence bearing on the question of the independent effects of dietary cholesterol, or its interaction with other nutrients, in influencing serum or lipoprotein cholesterol concentrations, cholesterol or lipoprotein metabolism, atherosclerosis, or atherosclerotic disease. As before, we have assumed that serum lipids are important intervening variables in the pathogenesis of atherosclerosis, and have not reviewed that issue. This review does not include a large number of reports that deal with the effects of fat-modified diets because the role of dietary cholesterol could not be isolated from that of other dietary components.

II. Assessment of Cholesterol Intake in the United States

Within-person variability.--McGee et al. (1982), as part of the Honolulu Heart Program, measured nutrient intake on 7 consecutive days in 329 men. There was a wide range of cholesterol intakes with a mean over the 7 days of 529 (183) mg/day. The means of the 329 men for different days differed significantly ($P<0.001$), ranging from 500 mg/day to 571 mg/day. The authors computed the coefficient of reliability, the percentage of variation attributable to between-person variability for each nutrient; and the within-person SD. Polyunsaturated fatty acids showed the lowest coefficient of reliability (0.34). Dietary cholesterol had next to the lowest (0.38), with a within-person SD of 253, about half the overall mean value. In contrast, the coefficient of reliability for starch was 0.66, and the within-person SD was 44, about one third the overall mean value. These results, the authors suggested, indicate that any correlations with dietary cholesterol intake based on a single 24-h recall would be attenuated to about two-thirds of their true sizes.

White et al. (1981) studied the problem of measuring the daily cholesterol intakes of individuals by training volunteers to keep food intake records and analyzing dummy diets. While consuming a self-selected low cholesterol diet for 20 days, intake calculated from dietary records was 144 (13) mg/day. This value was 19% higher than values based on chemical analyses, 118 (28) mg/day. The food intake records of 100 outpatients trained to adhere to a low cholesterol diet showed a mean daily intake of 251 mg/day, but there were large daily variations. The authors concluded that, with training in dietary record keeping, portion-size assessment, and adherence to a low cholesterol diet, nine days of dietary records provided a reliable quantitative estimate of cholesterol intake in free living populations.

Lee et al. (1985) compared the unquantified and quantified food-frequency interview methods for measuring cholesterol intake in 4,638 subjects. The two methods yielded intake values that had correlation coefficients of 0.36 for men and 0.30 for women. The unquantified frequency method gave higher intake values among low consumers, but the reverse was true for high consumers.

Summary and conclusions.--Dietary cholesterol continues to be one of the most difficult dietary components to measure accurately and reliably. There is a high degree of within-person variability from day to day. This variability would seriously degrade any correlations based on single 24-h dietary assessments, no matter how many subjects were involved.

Estimated cholesterol intake of U.S. population.--Ahrens and Boucher (1978) assessed the U.S. cholesterol intake by comparing the results of chemical analyses with estimates from food composition tables of a simulated American diet. The simulated diet was prepared from a USDA report prepared in 1968. The chemical analyses yielded a value of 576 mg/day while the food-table estimate yielded a value of 624 mg/day. Related to caloric intake, the daily cholesterol intake, based on chemical analyses, was 212 mg/1000 Kcal/day.

Abraham and Carroll (1981) analyzed cholesterol intake from data collected in the first National Health and Nutrition Examination Survey, which examined 20,749 persons by a 24-h recall and a food frequency questionnaire between 1971 and 1975. The results showed an overall mean cholesterol intake of 445 mg/day. The highest intake was in the 18-44 year age group, 521 mg/day. One-third of the cholesterol (35%) was derived from eggs.

Goor et al. (1985) reported the cholesterol intake measured in 4,568 white adults by 24-h recalls between 1972 and 1975. The data were collected as part of the Lipid Research Clinics Prevalence Study in nine North American

centers. The sample was not intended to be representative of the U.S. population and there were wide differences among the nine clinic groups. The mean dietary cholesterol intake for clinic groups of men varied from 380 to 590 mg/day, and for groups of women from 290 to 380 mg/day. Comparison of selected values with comparable previous reports showed a drop in cholesterol intake among men of about 10% from 10 years previously.

Fischer et al. (1985) computed dietary cholesterol intakes from the USDA 1977-1978 Nationwide Food Consumption Survey. This survey was based on three-day dietary records (24-h recall plus a 2-day record). The highest daily intakes were those for 19-34 year old men, 434 (316) mg/day; and the next highest were 35-50 year old men, 432 (299) mg/day. Women ingested less, 286 (237) mg/day for 19-34 year olds and 291 (222) mg/day for 35-50 year olds. Children under 5 years of age consumed 208 (134) mg/day; and those 6-11 years old, 281 (139) mg/day. When converted to mg cholesterol per 1000 Kcal, intakes of men and women were similar. For all of these mean intake values for adults, the standard deviations were nearly equal to the means, and the frequency distributions showed curves severely skewed to the high end. Consequently, many individuals were consuming more than 1000 mg/day of cholesterol.

Cholesterol intake in children.--Frank et al. (1985) assessed the cholesterol intake of 10-year old children in 1973-1974 in the Bogalusa Heart Study by 24-h recall, and repeated the measurement on the same children three years later. Overall mean intake on the survey of 10-year-olds was 324 (219), and of 13 year olds, 322 (237) mg/day. Relative to calories, cholesterol intake was 150 (82) and 137 (84) mg/1000 Kcal. The cholesterol intakes of both white and black boys was slightly higher than those of white and black girls, but, relative to calories, intakes of both sexes and both races were about the same.

Summary and conclusions.--Reports that appeared between 1981 and 1985 are remarkably similar in their estimates of cholesterol intake for the U.S. in the mid-1970's. The average intake for men was about 450 mg/day, and for women, about 300 mg/day. Intakes of men and women were similar when expressed relative to caloric intake, that is, about 200 mg/1000 Kcal, and were lower than the estimates of cholesterol intake of about 600 mg/day, or about 300 mg/1000 Kcal, for the decades before 1970. In all of these studies, between-person variability was high, and a few persons consumed 1000 mg/day or more. About one-third of dietary cholesterol was derived from eggs.

III. Correlations of Cholesterol Intake with Serum Lipid and Lipoprotein Levels

National Health and Nutrition Examination Survey.--Dietary intakes that were measured in NHANES I (Harlan, Series 11, No 227, 1983) were tested for their correlations with serum cholesterol levels. No association of cholesterol intake with serum cholesterol was found in any age group.

Lipid Research Clinics Prevention Trial.--In the recruitment phase of this trial, 6,494 hypercholesterolemic men were instructed in an iso-caloric diet containing 400 mg cholesterol per day and a P/S ratio of 0.8 (Gordon et al., 1982). Dietary intakes and plasma lipids and lipoproteins were measured one month before and one month after instruction. The instruction resulted in a decrease in cholesterol intake from 407 to 299 mg/day (174 to 147 mg/1000 Kcal), and a decrease in mean plasma cholesterol from 291 to 267 mg/dl, most of the 23 mg/dl decrease in plasma cholesterol was in LDL cholesterol (22 mg/dl). By multiple regression analysis, the authors demonstrated an effect on the change in plasma cholesterol level by the change in dietary cholesterol intake, independently of the other dietary changes. In a regression model computed to fit the observed data, the coefficient predicting the effect of a change in dietary cholesterol in mg/day was 0.015, which indicated that lowering dietary cholesterol by 100 mg/day would reduce plasma cholesterol by 1.5 mg/dl.

The Zutphen Study.--Kromhout analyzed dietary intake and serum cholesterol values measured in 1960, 1965, and 1970 on about 500 middle aged men in Zutphen, Netherlands (1983). Dietary intakes were assessed by the cross-check dietary history method. Changes in both serum cholesterol and dietary cholesterol were observed between the 5-year measurement intervals.

Change in dietary cholesterol, expressed as mg/1000 Kcal, was weakly but significantly correlated with the change in serum cholesterol for the 1970-1965 ($r=0.095$, $0.01<P<0.05$) and 1970-1960 ($r=0.127$, $0.001<P<0.01$) intervals.

Vegetarians.--Liebman and Bazzarre (1983) examined the relationships of egg consumption and dietary cholesterol intake in 36 vegetarians and 18 nonvegetarians. They found no significant correlations of plasma cholesterol concentrations with either dietary cholesterol intake or egg consumption within either group despite considerable variations in egg consumption.

Bogalusa Heart Study.--Frank et al. (1978) examined the cholesterol intake of 185 10-year old children who were part of the Bogalusa Heart Study, an epidemiologic survey in which multiple measurements of serum lipids and lipoproteins of children had been made. The intakes were computed from 24-h recall interviews adapted for children. The average cholesterol intake was 324 mg/day, or about 150 mg/1000 Kcal; the range of intake was from 0 to 1536 mg/day. Twenty six percent of the cholesterol was derived from eggs; 16%, from milk; and 13%, from beef. Simple correlation coefficients for dietary cholesterol and serum cholesterol, VLDL cholesterol, and HDL cholesterol were not statistically significant; for dietary cholesterol and LDL cholesterol, it was 0.171 ($P<0.05$).

Farris et al. (1982) examined the effects of milk source on nutrient intake and serum lipids and lipoproteins of about 350 infants at 6 months and 1 year of age. Six month old infants fed cow's milk ingested 130 (SE,34) mg cholesterol per day; those fed milk formula, 100 (SE,44) mg/day; and those fed soy formula, 87 (SE,72) mg/day. However, these differences were not statistically significant. The cow's milk group had slightly higher serum and LDL cholesterol levels than the other groups, but this difference could not be attributed to cholesterol intake alone because there also were considerable differences in the type of fat consumed.

Farris et al. (1984) analyzed the fat intakes of about 150 children 10 and 13 years old by 24-h recalls. For 10-year old children, the average cholesterol intake was 322 (237) mg/day, or 137 (84) mg/1000 Kcal. In 13-year old children, the average daily intake was 306 (213), or 129 mg/1000 Kcal. Cholesterol intakes of black children were slightly lower than those of white children. Comparison of the same cohorts studied at age 10 and again at age 13 showed moderate declines in cholesterol intake of all race and sex groups except white boys, whose intake remained at the same level. These cholesterol intake values were very similar to those reported by NHANES I (DHHS,PHS,1981).

Elderly persons.--Clarke et al. (1981) studied 91 elderly men and women (60 to 85 years) who participated in a nutrition program at meal site centers in Vermont. Dietary intakes were estimated by seven daily food intake records. Cholesterol intake was 422 mg/day for men; 261 mg/day for women. However, women had higher plasma cholesterol levels, and dietary cholesterol showed no correlation with plasma cholesterol.

Hooper et al. (1982) estimated food intakes of 270 free-living men and women (60 to 93 years) by 3-day food records. Cholesterol intake expressed relative to caloric intake showed no significant correlation with serum, HDL, or LDL cholesterol concentrations.

Yano et al. (1986) analyzed the relationship of dietary variables to plasma lipids and lipoproteins in 1965 men of Japanese ancestry over 60 years of age in the Honolulu Heart Study. The mean cholesterol intake was 319 (243) mg/day, or 171 (120) mg/1000 Kcal. There was no correlation of cholesterol intake with plasma, LDL, or HDL cholesterol concentrations.

Polynesian population.--Darlu et al. (1984) surveyed the inhabitants of five villages on Tahuata Island, one of the Marquesas Islands in Polynesia. Among the villages, average cholesterol intakes ranged from 227 to 352 mg/day

for men and from 184 to 366 mg/day for women. The differences among villages were significant, and the differences between men and women also were significant. The serum cholesterol levels were not significantly different among villages for men, but they were different among villages for women. There was no correlation between the mean cholesterol intakes and mean serum cholesterol levels.

US and USSR populations.--The Lipid Research Clinics staff (1984) analyzed data collected in the Joint US-USSR LRC Program Prevalence Study from nine centers in the US (1,029 men) and two in the USSR (692 men). Nutrient intake was assessed by single 24-h recalls. Daily U.S. cholesterol intake was 468 (308) mg/day or 188 (123) mg/1000 Kcal; USSR, 508 (317) mg/day, or 199 (111) mg/1000 Kcal. Correlation coefficients for dietary cholesterol as mg/1000 Kcal with LDL and HDL cholesterol concentrations were very small and were not statistically significant. Dietary cholesterol did not emerge as a predictor of LDL or HDL cholesterol in stepwise multiple regression analysis.

Intensive diet records.--Church et al. (1984) collected dietary records for 365 days on 29 free-living subjects 20 to 53 years of age (13 men, 16 women) and measured serum and HDL cholesterol five times during that period. The mean cholesterol intake for the year for all subjects was 373 mg/day, or 172 mg/1000 Kcal. The overall mean serum cholesterol was 204 (SE,3) mg/dl. Neither serum cholesterol nor HDL cholesterol was correlated with cholesterol intake.

Summary and conclusions.--As was noted in the 1979 review (McGill, 1979), it is nearly impossible to demonstrate associations between dietary cholesterol and serum cholesterol or lipoprotein cholesterol concentrations in cross sectional studies of a population. The reasons for this failure, despite the effects of dietary cholesterol that are consistently demonstrated

in experiments in which dietary cholesterol is altered in formula or controlled diets, have been examined in a recent review (McGill, 1981), an editorial (Blackburn and Jacobs, 1984), and a statistical analysis of the effect of dietary variability on associations of nutrition with disease or physiological variables (el Lozy, 1983). Additional studies of this type appear to be of little value in view of the many results that are negative or show weak associations of marginal significance, and also in view of the theoretical reasons why an association would be difficult to detect if it were present.

IV. Effects of Changing Dietary Cholesterol Intake

Removal and addition of eggs.--Flynn et al (1979) studied 116 free living men, ages 32 to 62 years, who ate their customary diets without eggs for 12 weeks and the same diet with two eggs added for 12 weeks. Four day food records in each diet period were used to assess nutrient intake, and a Latin square design allowed analysis for seasonal and carryover effects. The addition of two eggs increased the daily cholesterol intake from 260 (10)mg to 800 (15)mg. Total fat and saturated fat intakes were slightly greater during the egg diet period. There were no significant effects of egg consumption or season or serum cholesterol or triglyceride levels. There was a high degree of individual variability within and between individuals in serum cholesterol levels. In a subsequent letter, Sacks (1983) commented that "the dietary changes that occurred in the subjects did not produce the anticipated changes in the nutrient composition of the diet", suggesting that the experiment was concerned more with practical dietetics than with the biologic effects of dietary cholesterol.

Apo B and LDL cholesterol.--Applebaum-Bowden et al. (1979) studied four subjects after consuming a liquid formula diet containing 5000 mg of egg yolk cholesterol per day for 30 days. On this diet, plasma cholesterol increased in all four subjects by an average of 33 mg/dl ($P<0.05$), and plasma apo B levels increased in about the same proportion. Most of this increase (28 mg/dl) was in LDL cholesterol. VLDL and IDL cholesterol did not increase in any consistent manner. HDL cholesterol and apo A-I increased slightly but not significantly on the high cholesterol diet.

Flain et al. (1981) compared two groups, each made up of 12 young men that had been matched for plasma cholesterol concentrations on their customary

diets. One group was fed a diet containing about 400 mg cholesterol per day; the other group, a diet closely matched for energy, protein, and fat but containing about 1400 mg cholesterol per day derived from 4 added whole eggs. The diet period was 4 weeks. There were no significant changes in plasma cholesterol, or in LDL or HDL cholesterol. Despite the lack of overall average significant change, two of the 12 subjects on the high cholesterol diet showed substantial (value not specified) and persistent elevations.

Removal of eggs.--Bronsgeest-Schoute et al. (1979c) tested the removal of eggs from the diet of 44 free-living persons (25 males, 19 females) who habitually consumed seven or more eggs per week. The habitual intake of cholesterol was estimated to be 742 (SE,60) mg/day; the test intake, 264 (SE,13) mg/day. Serum cholesterol fell after 3 weeks by 6 (16) mg/dl ($P<0.05$). Individual responses ranged from +20 to -50 mg/dl. Seven of the 44 subjects showed decreases between 20 and 50 mg/dl; the remaining 37 had an average decrease of only 1 mg/dl. There was no relationship of the response to obesity, age, or initial serum cholesterol level.

Double blind experiment.--Roberts et al. (1981) compared the effects of whole eggs with those of an egg substitute in 16 outpatients (8 men, 8 women) 22 to 61 years of age in an attempt to conduct a double blind experiment. Each diet period was 4 weeks, and the diets were assigned in a crossover design. Two eggs, or egg substitute equivalents, prepared so as to be indistinguishable, were used by the subjects in their homes. The average serum cholesterol concentration after 4 weeks of eggs was 243 (39) mg/dl; after the egg substitutes, 219 (44) mg/dl ($P<0.01$). As indicated by the standard deviations, variability in response was high, and about half the subjects were low responders.

Lipoproteins and LDL receptor activity.--Mistry et al. (1981) fed 37 healthy medical students and laboratory personnel (19 to 47 years; mean, 24 yrs) 6 egg yolks per day for 14 days, equal to about 1,500 mg cholesterol more than their habitual intake of 522 mg/day. These subjects experienced a mean elevation of plasma cholesterol of 29 mg/dl ($P<0.001$), but individual responses ranged from a decrease of 6 mg/dl in two subjects to an increase of 75 mg/dl in one. In 12 subjects tested 38 days after cessation of egg yolk feeding, plasma cholesterol had returned to baseline levels. Another 14 subjects (19 to 60 yrs; mean, 30 yrs) consumed 3 egg yolks per day for 28 days and experienced a similar rise in mean plasma cholesterol of 24 mg/dl ($P<0.001$), but the authors did not provide information on individual variability among the second group. About 60% of the increased plasma cholesterol was contained in LDL; the rest, in IDL and HDL_2. Apo B also increased proportionately. Freshly isolated mononuclear cells from peripheral blood showed a 17% increase in cholesterol content and a 32% ($P<0.001$) reduction in HMG-CoA reductase activity. LDL receptor activity was reduced by 74% ($P<0.01$). The percentage of increase in LDL cholesterol was inversely correlated with HMG-CoA reductase activity.

Cholesterol effects on lipoproteins.--Schoenfeld et al. (1982) studied 20 young healthy men, 22-31 years old, with two levels of cholesterol as 3 or 6 eggs daily (750 or 1500 mg cholesterol) added to their habitual intakes of 500 to 700 mg per day for 4 to 6 weeks. The two levels of cholesterol were fed with four types of dietary fat with a P/S ratios of 0.25, 0.4, 0.8, or 2.5. With P/S ratios of 0.25 and 0.4, addition of 750 mg cholesterol elevated LDL cholesterol by 16 (14) mg/dl ($P<0.01$); 1,500 mg increased it by 25 (19) mg/dl. With a P/S ratio of 0.8, 75 mg produced no significant changes, but 1,500 mg increased LDL cholesterol by 17 (22) mg/dl ($P<0.02$). With the P/S

ratio of 2.5, neither dose of cholesterol changed LDL cholesterol. Thus, there appeared to be an interaction of dietary cholesterol with saturated fat, so that the cholesterolemic effect of dietary cholesterol was greater with saturated fat than with polyunsaturated fat. However, no specific statistical analyses to test for an interaction were performed.

With each type of fat, elevations in LDL cholesterol varied greatly among individuals. With a P/S ratio of 0.25 and 750 mg added cholesterol, the increases ranged from 2 to 44 mg/dl; while with 1,500 mg, the increases ranged from 8 to 62 mg/dl. Similar variation was seen with the other diet combinations.

Extensive analyses of lipoproteins disclosed that the changes in plasma cholesterol concentration were predominantly in LDL cholesterol. There were no significant effects of dietary cholesterol on HDL cholesterol. Apo B changes corresponded to the LDL cholesterol levels. There were no substantial or significant changes in other lipids or apoproteins; or in the composition, flotation rates, molecular weights, or binding of LDL by cellular receptors.

Fisher et al. (1983) studied intensively the responses of 9 young normolipidemic males, aged 18 to 37 years, to saturation of dietary fat and dietary cholesterol in a 2 x 2 design. The fats were corn oil and coconut oil, fed as 31% of calories; and cholesterol was added as 1 g U.S.P. cholesterol per day for the high cholesterol diet. The cholesterol content of the low cholesterol diet was not given, but presumably was negligible. Each formula diet was fed for 9 days, and blood samples were taken on each of the last 3 days of each period. Extensive analyses of plasma lipids and lipoproteins and of apo E were performed. The type of fat affected almost every variable measured, but dietary cholesterol produced a significant effect only on VLDL apo E. There were no interactions between dietary cholesterol and type of fat in their effects on major lipoprotein classes.

Apo B metabolism and LDL receptor activity.--Packard et al. (1983) studied seven healthy normolipidemic volunteers (21 to 28 years; three men, four women) on a control diet containing 180 (110) mg cholesterol per day, and after four weeks of a similar diet to which 6 eggs were added. This supplement raised the daily cholesterol intake to 1,470 (80) mg. The P/S ratio remained constant at 0.17, and the percent of calories from fat also remained constant at 37%. The added dietary cholesterol increased plasma cholesterol from 197 to 254 mg/dl, a difference of 57 mg/dl ($P<0.01$). Of the elevation, 48 mg/dl were in LDL cholesterol and 11 mg/dl were in HDL (VLDL cholesterol fell by 2 mg/dl). The increases in LDL cholesterol varied among individuals from 12 mg/dl to 77 mg/dl. Apo B was also increased proportionately to LDL cholesterol. The composition of LDL was not altered. Kinetic studies showed that there was a 23% increase in synthesis of apo B, and a 10% fall in the fractional removal rate of LDL. Receptor independent removal remained unchanged, and it was presumed that LDL receptor activity was reduced. The authors did not attempt to relate response of LDL cholesterol to the individual kinetic values.

Eggs and vegetarians.--Masarei et al. (1984) examined the effects of a lacto-ovovegetarian diet on a number of plasma lipids, lipoproteins, and apolipoproteins in 36 subjects, and used simple correlation coefficients and principal component/multiple regression analysis to isolate the effects of various dietary components. Changes in dietary cholesterol, which were relatively small and within the 300 to 500 mg/day range, showed no effect on plasma cholesterol.

Sacks et al. (1984) fed 17 lactovegetarian students (18 to 24 years; 4 men, 13 women) a diet supplement with and without one extra egg per day, each for 3 weeks. The supplement was double blind. Dietary cholesterol intake was

increased from 97 to 418 mg per day. Plasma LDL cholesterol rose by 12 mg/dl (P=0.005) and apo B rose proportionately. There were no significant changes in other plasma lipids, lipoproteins, or apoproteins.

Summary and conclusions.--It remains difficult, but not impossible, to demonstrate effects of dietary cholesterol on serum or lipoprotein cholesterol levels in free-living persons by asking them to alter their egg consumption. Undoubtedly, experiments of this type suffer more from lack of adherence to protocol and fluctuations caused by other environmental variables than do experiments conducted in an institutional setting, or experiments in which diets are prepared in a central kitchen. One cannot prove that responsiveness to dietary cholesterol does not exist by conducting experiments with free-living persons unless dietary intakes are rigorously controlled or measured, and unless other dietary variables are also controlled, when so many controlled studies show an effect. Unless new egg substitute food products, dietetic counseling techniques, or other measures designed to affect the dietary cholesterol intakes of free-living persons are to be tested, there seems to be little value in conducting more studies of this type.

The better controlled studies conducted since 1979 have extended considerably the evidence indicating that dietary cholesterol elevate serum cholesterol. They have further shown that the additional cholesterol is carried mainly in an increased number of normal LDL particles. Changes in other lipoproteins were occasionally reported, but not as consistently as were changes in LDL. Changes in serum apo B concentrations paralleled the changes in LDL.

Although not directed specifically at determining the frequency distribution of responses to dietary cholesterol, nearly all investigators report individual values and comment on the wide variability in response. None of these studies specifically tested the reproducibility of the response.

One experiment fed cholesterol with fats differing in saturation of fatty acids, and the results suggested an interaction of dietary cholesterol with saturated fat in their effects on plasma and LDL cholesterol. However, the design of the experiment did not permit a specific statistical analysis for interactions.

There was much hope for and speculation about the possibility of an effect of dietary cholesterol on plasma lipoproteins other than the plasma concentrations. This hope was stimulated by the oft quoted report of Mahley et al. (1978) that egg feeding increased the ability of a subfraction of HDL to bind to cell surface receptors of fibroblasts. Unfortunately, this observation has not been confirmed, and no other similar mechanism has been suggested by the published reports.

V. Predictive Equations Relating Dietary Cholesterol to Serum Cholesterol

Keys (1984) and Hegsted (1986) presented updated analyses of the relationship of dietary cholesterol to serum cholesterol. Both had published equations describing this relationship previously, both developed new equations based on reports of experiments in which the effects of dietary cholesterol could be isolated. After reviewing 39 reports, Key concluded that the best equation was

$$\Delta \text{ Cholesterol} = 1.5\ (X_2^{\frac{1}{2}} - X_1^{\frac{1}{2}}), \qquad (1)$$

where Δ cholesterol is the change in serum cholesterol in mg/dl, X_2 is the new intake of cholesterol in mg per 1000 Kcal, and X_1 is the previous intake of cholesterol in the same units. This equation predicts that halving the intake of cholesterol when it is 300 mg/1000 Kcal is expected to reduce serum cholesterol by 7.6 mg/dl.

Hegsted reviewed the same 39 reports plus several additional values and called attention to the large differences in serum cholesterol response that had been reported for comparable changes in dietary cholesterol. He also described the many factors that make estimation of a response very difficult, particularly between-subject variation in response, and within-subject variation in serum cholesterol even when diet is constant. Hegsted presented four equations, derived from the published response data, that explained by regression between 87.9% and 94.4% of the variance. The simplest, which accounted for 94.2% of the variance, was

$$\Delta \text{ Cholesterol} = 0.0974X, \qquad (2)$$

in which X is the change in dietary cholesterol in mg/1000 Kcal. This linear equation fits the data well within the range of cholesterol intakes usually considered, that is, from 0 to 400 mg/1000 Kcal. This equation predicts that,

within this range, each mg/1000 Kcal increases serum cholesterol by about 0.1 mg/dl. The 150 mg/1000 Kcal decrease (halving an intake of 300 mg/1000 Kcal) that Keys predicted would decrease serum cholesterol by 7.6 mg/dl would, by Hegsted's equation, decrease serum cholesterol by 15 mg/dl.

Whichever is the more nearly correct, the important answer is clear: on the average, changes in dietary cholesterol within the ranges ordinarily consumed by humans in the industrialized countries have a definite, but small, effect on serum cholesterol concentrations in experiments that change cholesterol intake.

VI. Identification of High and Low Responders to Dietary Cholesterol

Concept of inherent variability in response.--In the 1979 review (McGill, 1979), we suggested that there appeared to be a high degree of variability in the sensitivity of individuals to dietary cholesterol, and that differences in the balance of responders and nonresponders might account, in part, for some of the variability among experiments in the results and conclusions. Furthermore, if there were considerable variability, and if this variability were a characteristic and reasonably constant feature of each person, the frequency distribution of responders would be important information for the design of dietary recommendations. Reports of experiments with dietary cholesterol before 1980 had rarely reported more than means and overall indicators of variation such as standard deviations or standard errors. These generally showed high variability in response, but gave no indication of the shape of the frequency distribution. After 1980, investigators began either to report individual values in experiments, or to report the range of responses. Some reports presented frequency distributions of the responses, and also tested the reproducibility of the response. Reproducibility was an important issue because of the well known fluctuations in plasma and lipoprotein cholesterol levels, even under constant dietary and other environmental conditions.

Jacobs et al. (1983), in order to assess variability in response to both type of fat and amount of dietary cholesterol, reanalyzed data from a number of dietary experiments conducted by Ancel Keys and his associates at the University of Minnesota between 1963 and 1966. It was not possible to isolate the effects of dietary cholesterol from those of fat, but multiple measurements on each person minimized the effects of day-to-day variability.

A response score was computed for each individual by comparing the response predicted by an equation developed previously and the individual's actual response. Of 58 men, five (9%) were considered to be essentially nonresponders, 82% responded to an intermediate degree, and five (9%) were high responders.

Classification of levels of response.--Oh and Miller (1985) classified 21 subjects as hyper- and hypo-responders on the basis of their responses to eating 3 additional eggs per day, raising their cholesterol intake from an average of 474 mg/day to about 1100 mg/day. Plasma cholesterol rose from 188 (37) to 199 (36) mg/dl (NS). Eight subjects had elevations of plasma cholesterol of 8% or more, and these were classified as hyper-responders; while 13 had responses less than 5%, and were classified as hypo-responders. The 13 hypo-responders were than fed 6 eggs per day for an additional 6 weeks. Their mean plasma cholesterol then rose to 211 (27) mg/dl, an increase of 12 mg/dl ($P<0.05$). Six of the putative hypo-responders showed small elevations of plasma cholesterol during this period, and seven showed no change. Most of the additional cholesterol in the hyper-responders was in LDL. Since the classification at the end of the first feeding period was based on only one blood sample, it is not surprising that several of the subjects might have been misclassified because of the well known fluctuations in plasma cholesterol levels, even on a constant diet.

Reproducibility of response.--Beynen and Katan (1985a) re-tested in 1982 the responses to egg yolk feeding of 34 healthy men and women, ages 23 to 78 years, who had been tested for a similar response in 1976. "Response" was defined as the change in serum cholesterol at 3 weeks after reduction of daily cholesterol intake from 800 to 300 mg/day. Overall average decline in serum cholesterol concentration was 6 (16) mg/dl in 1976, and 12 (14) in 1982. The

range of individual changes was from -39 to +19 mg/dl. The correlation coefficient between 1976 and 1982 responses was 0.32 ($P<0.05$). Four consistently low responders and two consistently high responders were tested again and their responses compared with cholesterol intakes of 116 mg/day and 640 mg/day. The four putative low responders decreased in serum cholesterol of 4.3 mg/dl, while the two putative high responders decreased by 19.7 mg/dl. The authors concluded that there were consistent and repeatable differences in the responses of individuals to dietary cholesterol.

Beynen and Katan (1985b) studied six young normolipidemic subjects (26-42 years; 3 men, 3 women) on a diet containing 207 mg cholesterol per day for 10 days and again on an egg-supplemented diet containing about 1800 mg cholesterol per day for 10 days. Serum cholesterol rose by 13%, 90% of which was contained in LDL. The experiment was repeated a year later and yielded similar results. There were also increases in HDL_1 and HDL_2 upon cholesterol feeding, but no change in HDL_3.

Katan et al. (1986) (also reported partially by Beynen and Katan, 1985c) conducted the most thorough test of the consistency of response to dietary cholesterol in humans. They tested 94 persons (46 men and 48 women; average age 33) on diets providing 121 and 625 mg cholesterol per day, the added cholesterol derived from egg yolks. One meal was served in a central dining room on week days, and other foods were distributed from a central source. Amount of fat was maintained at 41 to 42% of calories, and the P/S ratio was maintained at 0.16. Blood was collected on two consecutive days after two weeks of each diet. After the high cholesterol diet, serum cholesterol rose, on the average, by 19 (15) mg/dl. The response showed a normal Gaussian distribution.

Of these 94 individuals, the investigators identified 17 high responders (from the highest quartile) and 15 low responders (from the lowest quintile). These 32 subjects participated in two followup experiments which repeated the dietary challenge one month and again seven months after the completion of the initial test. Cholesterol intake in the second experiment was 673 mg/day, about the same as in the first experiment; but was increased to 989 mg/day in the third experiment. The 17 high responders showed increases of 11 (15) mg/dl in the second experiment and of 32 (14) mg/dl in the third experiment. The 15 low responders showed increases of 2 (14) mg/dl in the second experiment and of 18 (10) mg/dl in the third experiment. The high and low responding groups were significantly different from one another in the two follow up experiments.

Standardized regression coefficients of the serum cholesterol response to dietary cholesterol in pairs of the three experiments ranged from 0.34 to 0.53 (all $P<0.05$). The corrected between-subject standard deviation of response to about 500 mg of added dietary cholesterol was 11 mg/dl; the within-subject standard deviation also was estimated to be 11 mg/dl. Using the mean of each subject's responses during the three experiments as the best estimate of his response, frequency distribution showed a normal, unimodal distribution of values ranging from -12 to +46 mg/dl. The authors concluded that "modest differences in responsiveness of serum cholesterol to dietary cholesterol do exist in man, and that the wide scatter of responses observed in single experiments is largely due to chance fluctuations."

Summary and conclusions.--The existence of consistent and reproducible variations among individuals in responsiveness to dietary cholesterol, as measured by changes in plasma cholesterol, LDL cholesterol, or apo B concentrations, appears to be well established. However, because of the

relatively large day to day fluctuations in these variables, reliable measurement of an individual's responsiveness is quite difficult. No simple, discrete marker for sensitivity is available. Further study of the mechanisms involved in determining sensitivity, and eventually developing a rapid, inexpensive method of detecting sensitivity without the cumbersome dietary challenge and multiple plasma analyses, should be an important research objective.

We now have some evidence regarding the frequency distribution of responders and nonresponders. Responsiveness has a unimodal distribution. The definition of a responder, or a high responder, is arbitrary, but it appears that between 10 and 20% of adults are likely to be high responders--that is, their response will be greater than that predicted by the Keys (1984) or Hegsted (1986) equations. The elevation of serum or LDL cholesterol by this degree, if maintained over several years, would be widely accepted as conferring increased risk of atherosclerotic disease.

VII. Metabolic Processes Involved in Response to Dietary Cholesterol

Cholesterol absorption.--Simons et al. (1978) measured the absorption of cholesterol in vegetarian and non-vegetarian Seventh Day Adventists, and found no relationship between cholesterol absorbed and plasma cholesterol concentration.

Cholesterol biosynthesis and excretion.--Lin and Connor (1980) studied intensively the responses to dietary cholesterol of two hospitalized subjects over a 5-month period. One was a 67 year old type II a hypercholesterolemic woman; the other a 31 year old healthy normocholesterolemic man. Formula diets provided contrasting daily intakes of cholesterol of 45 mg (low) and 1000 mg (high). The woman's average serum cholesterol on the low cholesterol diet was 280 (7) mg/dl; on the high cholesterol diet, 427 (15) mg/dl, a difference of 147 mg/dl. The man's average serum cholesterol on the low cholesterol diet was 123 (6) mg/dl on the high cholesterol diet, 166 (7) mg/dl, a difference of 43 mg/dl. The major part of these increases were in LDL cholesterol, but HDL cholesterol also increased. Percent cholesterol absorption on the high cholesterol diet was unchanged from the percent absorption measured in another experiment in which cholesterol intake was low. Cholesterol biosynthesis decreased and bile acid excretion increased during high cholesterol feeding. These results demonstrated at least two compensatory mechanisms against cholesterol overloading, but, because of the limited number of subjects, few generalizations can be made. One subject illustrated an extreme high responder to dietary cholesterol.

Apo B metabolism.--Ginsberg et al. (1981) tested five young normocholesterolic men (32 to 35 years) only two diets, one of which provided 150 mg cholesterol per 1000 Kcal, and the other, 500 mg per 1000 Kcal. The

excess cholesterol was derived from egg yolk. Type and amount of fat was kept constant. The diets were fed 4 to 5 weeks. These five subjects turned out to be almost completely nonresponsive to dietary cholesterol, since there were no changes in plasma, VLDL or LDL cholesterol. Furthermore, there were no changes in rates of production or clearance of apo B in VLDL or LDL.

LDL receptor activity.--Applebaum-Bowden et al. (1984) studied 9 young healthy adults (20 to 33 years; six men, three women) to determine the effects of dietary cholesterol on LDL receptor activity in mononuclear blood cells. The subjects consumed two diets, one containing 137 mg cholesterol per day, and the other, 1034 mg cholesterol per day, each for one month in a cross-over design. The average plasma cholesterol increased by 11 mg/dl, all of which was in LDL ($P<0.02$). Apo B levels were not significantly changed. LDL receptor activity in mononuclear cells, measured by their ability to degrade ^{125}I-labeled LDL, decreased by 41% ($P<0.05$) after cholesterol feeding. The reduction in LDL receptor activity was associated with the rise in LDL cholesterol ($r= -0.796$, $P=0.06$).

Characteristics of responders and nonresponders.--Katan and Beynen (In press) compared characteristics of the 32 high and low responders that had been identified previously (Katan et al., 1986). There was no relationship of responsiveness to age, sex, caloric needs, or the response of endogenous cholesterol synthesis to dietary cholesterol. Responsiveness was correlated negatively with habitual cholesterol consumption ($r = -0.62$), body mass index ($r = -0.50$), and endogenous cholesterol synthesis ($r = -0.40$). Responsiveness was correlated positively with HDL_2 cholesterol level ($r = 0.41$) and with serum cholesterol level on the high cholesterol diet ($r = 0.31$). With multiple regression analysis, only habitual cholesterol intake and serum and HDL_2 cholesterol levels contributed significantly to explaining variation in response.

Summary and conclusions.--Absorption of dietary cholesterol does not appear to be the process that determines an individual's responsiveness. Before absorption is rejected as a possible mechanism, however, it should be noted that it is difficult to measure cholesterol absorption precisely, and measurement error may have obscured a relationship. LDL receptor activity decreases as the LDL cholesterol level increases, but the decline in receptor activity may be secondary to the rise in plasma LDL. The negative correlation with previous levels of intake cholesterol suggests the possibility of adaptation to cholesterol intake, such as has been observed in animals (McGill, 1981). With the knowledge of the molecular biology of lipoprotein metabolism now available, there should be a number of new hypotheses to test in searching for the physiological basis, whether genetic, acquired, or both, of the variation in sensitivity to dietary cholesterol.

VIII. Interaction of Dietary Cholesterol with other Nutrients and Conditions

Fish oil.--Nestel (1986) measured serum and lipoprotein cholesterol in six young normolipidemic subjects (sex not specified) consuming their habitual diets and consuming, for 3 weeks, similar diets in which 40 g fish oil per day were substituted for fat. One fish oil diet contained 190 mg cholesterol per day; the second, 940 mg cholesterol per day derived from egg yolk. Changing to the fish oil diet resulted in lowering of plasma cholesterol concentration by 42 mg/dl ($P<0.001$) and lowering of all lipoprotein cholesterol fractions measured -- VLDL, LDL, and HDL, and all apoproteins. However, adding 750 mg cholesterol to the fish oil diet did not cause significant elevation of plasma cholesterol, any of the lipoprotein cholesterol fractions, or apoproteins. The author concluded that fish oil, rich in N-3 fatty acids, suppressed the dietary cholesterol-induced rise in LDL cholesterol.

Linoleic acid.--Bronsgeest-Schoute et al. (1979a) examined the interaction of dietary cholesterol with linoleic acid in a cross-over design with 41 healthy young men and women. Cholesterol intakes were about 200 mg/day and 600 mg/day, with the added cholesterol derived from two egg yolks. Linoleic acid was provided at about 3% and 14% calories with the additional linoleic acid derived from margarines and food oils. Each diet was consumed for two weeks. The additional cholesterol with high linoleic acid elevated serum cholesterol by 11 (11) mg/dl ($P<0.001$), approximately the expected rise. Thus, linoleic acid did not attenuate the cholesterol-induced rise in plasma cholesterol.

In another experiment with 18 subjects, Bronsgeest-Schoute et al. (1979) tested the effects of dietary cholesterol with a linoleic acid-poor diet in which this fatty acid provided only 5% of the calories. Three-week feeding

periods were used. Adding two eggs per day elevated serum cholesterol by 26 (20) mg/dl ($P<0.01$). Correction of the elevations estimated to be due to differences in dietary fat resulted in an elevation of 21 mg/dl. The elevation encountered here was greater than that found in the previous experiment (Bronsgeest-Schoute et al., 1979a) with high linoleic acid diets, and this difference is interpreted by the authors as indicating an interaction of dietary cholesterol with saturated fat. However, interpreting these findings as in interaction with the many differences between the subjects and the experiments appears to be hazardous.

Type and amount of fat.--Chenoweth et al (1981) recruited 32 young men who habitually ate two eggs per day, and who had high serum cholesterol levels (range 192 to 323 mg/dl; mean, 227 mg/dl), as subjects. An experiment was conducted to determine the effects of two added eggs daily, either combined with a high saturated fat diet (42-45% calories, P/S = 0.3-0.5) or with a modified fat diet 32-35% calories; P/S $\geq$ 1.0). As a control dietary component for the eggs, a cholesterol-free egg substitute was used to maintain other nutrients at a constant level. Half the subjects received the high saturated fat basal diet with and without added cholesterol; the other half, the modified fat basal diet. A crossover design was used with each group. The feeding period for each test diet was 4 weeks. With the high saturated fat basal diet, the average change (increase or decrease) in serum cholesterol asscciated with two eggs daily was about 20 mg/dl. With the modified fat diet, the average change in serum cholesterol was about 14 mg/dl. The dietary cholesterol effects were statistically signficant, ($P\leq 0.05$), and analysis of variance showed no evidence of an interaction of dietary cholesterol with type of fat. As in all experiments of this type, there were wide variations among subjects in dietary responses from -10 to +65 mg/dl. These results, it should be noted, were obtained in persons who are likely to have been high responders.

Oh et al. (1985) used 11 young normolipidemic men in a 2x2 factorial cross-over design with two levels of dietary cholesterol (300 and 1000 mg/day) and two types of fat (P/S, 0.28 and 1.8). A 12-week feeding period was used. Each group of 2 or 3 subjects was sequentially placed on two different diets differing only in P/S ratio; all subjects received both the high and low cholesterol diets. The results were analyzed with emphasis on the type of fat effects rather than on dietary cholesterol effects, but inspection of the data indicates that dietary cholesterol independently elevated plasma cholesterol with both types of fat, and that there was no interaction between cholesterol and type of fat.

Reggiani et al. (1984) compared serum and HDL cholesterol levels in highly active young women athletes with those in inactive age-matched women, and also estimated nutrient intake by 7-day food records. The active women consumed considerably more cholesterol than did the inactive ones. The active group had significantly higher HDL cholesterol levels, but there was no correlation between dietary cholesterol intake and HDL cholesterol. No analysis to test for an interaction between dietary cholesterol and physical activity was reported.

Fiber.--Raymond et al. (1977) tested the effects on cholesterol metabolism of large volumes of dietary fiber on 6 adult subjects fed eucaloric cholesterol-free formula diets. The added fiber did not change plasma cholesterol LDL or HDL cholesterol concentration, or bile acid excretion, but did increase fecal steroid excretion and decreased intestinal transit time. Another group of six subjects (including four from the first group) were fed added fiber with a similar formula containing 1,000 mg cholesterol per day derived from egg yolk. Fiber again did not alter plasma cholesterol, lipoprotein cholesterol, bile acid excretion, total fecal steroids, or

absorption of dietary cholesterol. Using the plasma lipid values from all eight subjects, analysis of variance showed a highly significant effect on plasma cholesterol concentration (values not reported), but no interaction between fiber and dietary cholesterol.

Ascorbic acid.--Buzzard et al. (1982) examined the interaction of dietary cholesterol with ascorbic acid in their effects on plasma and lipoprotein cholesterol. Forty healthy free-living young men were divided into four groups, matched for plasma cholesterol levels. Each group consumed a different diet so that there were two levels of cholesterol intake, about 400 and 1000 mg/day, with the added cholesterol derived from 3 eggs; and two levels of ascorbic acid intake provided by adding 2 g ascorbic acid per day. After six weeks of the diets, there were no significant changes of plasma cholesterol in the high cholesterol-low ascorbic acid or low cholesterol-high ascorbic acid groups. The high cholesterol-high ascorbic acid group showed a significant increase in plasma cholesterol of 18 mg/dl and in LDL cholesterol of 10 mg/dl. In the high cholesterol-low ascorbic acid group, four of the 10 subjects showed substantial responses and six showed a minimal response or a decline. Analysis of variance showed a significant interaction between the effects of dietary cholesterol and ascorbic acid.

Physical activity -Hartung et al. (1980) investigated the relationship of diet to total and HDL cholesterol concentrations in 218 men with three contrasting levels of physical activity--marathon runners, joggers, and inactive men. Egg consumption was not correlated with serum cholesterol concentration by multiple correlation coefficients, but it was related to HDL cholesterol concentration by multiple correlation coefficients ($r=.564$, $P<0.001$) and by stepwise multiple regression analysis ($R^2=.318$, $P<0.001$).

There was no interaction of egg consumption with activity in their effects on HDL cholesterol. This result is surprising, especially since the overall average egg intake was only about 3 eggs per week.

Quig et al. (1983) followed plasma and lipoprotein cholesterol concentrations in 23 sedentary young men during 4 weeks of controlled feeding and 6 weeks of aerobic conditioning. The subjects consumed diets, each with a P/S ratio of about 0.6, and either 400 or 1400 mg cholesterol per day derived from egg yolks. High dietary cholesterol elevated plasma cholesterol in the sedentary groups by 32 mg/dl ($P<0.05$), but in the exercise group by only 11 mg/dl (NS). Combined over both exercise groups, the added cholesterol elevated plasma cholesterol by 17 mg/dl ($P<0.05$). As with other experiments, a high degree of individual variation in responses was observed. There were changes in VLDL, LDL, and HDL cholesterol, generally in the same direction as plasma cholesterol, but none of these was significant.

Tarahumara Indians.--McMurray et al. (1982) tested the responses to dietary cholesterol of 8 Tarhumara Indians. These Indians had been found previously to have very low plasma cholesterol levels (120 mg/dl), presumably due to their very low fat, low cholesterol diet. After 3 weeks of a cholesterol-free diet, their average plasma cholesterol concentration was 113 mg/dl. After 3 weeks of a high cholesterol diet (with identical fat content, 20% of calories) containing 1000 mg cholesterol per day (derived from egg yolk), plasma cholesterol rose to 147 mg/dl ($P<0.01$). The range of increases was from 18 to 57 mg/dl. Most of the elevated plasma cholesterol was in LDL cholesterol.

Eight Tarahumara Indians (apparently the same subjects) were studied further (McMurray et al., 1985) to determine the effects of dietary cholesterol on cholesterol metabolism. Nine hundred mg cholesterol per day

(1000 mg was the amount described in the previous report) did not affect percent cholesterol absorption, which was about 27% on both high and low cholesterol diets. Fecal sterol excretion increased greatly, primarily of unabsorbed cholesterol. Estimated cholesterol biosynthesis decreased from 755 (50) mg/day during the cholesterol-free diet period to 391 (66) mg/day during the high cholesterol diet ($P<0.001$). The decreased synthesis made the cholesterol input almost equal for the two diet periods. Although the percent absorption of dietary cholesterol among these subjects was considerably less than among caucasians in industrialized societies, their other responses to dietary cholesterol appeared to be similar.

Familial hyperchlesterolemic heterozygotes.--Stein et al. (1982) studied 12 FHC heterozygotes and 11 of their normal healthy siblings between 6 and 19 years of age while consuming four diets with two levels of cholesterol intake (less than 160 and more than 450 mg/day) and with varying types and amounts of fat (less than 35% of calories, P/S>1.5; and more than 40% of calories, P/S=1 or less than 0.4). Reduction in cholesterol intake while the FHC children were consuming the high P/S fat diet resulted in a slight but significant reduction in plasma and LDL cholesterol. Correlation coefficients relating the change in cholesterol intake to the change in plasma cholesterol ranged from -0.003 to 0.61. Several of the higher correlation coefficients were statistically significant. The correlations for dietary cholesterol with plasma cholesterol were higher in the FHC subjects than in the normal subjects. HDL cholesterol showed no significant changes. The design did not permit a precise estimate of the independent effects of dietary cholesterol or of a possible interaction between cholesterol and type of fat because types and amounts of fat were not held to two contrasting levels.

Cole et al. (1985) fed four FHC heterozygous subjects diets low in fat and cholesterol (271 mg/day), high in fat, and high in fat and cholesterol (750 to 1500 mg/day), each for 6 weeks. The increased dietary fat abone increased serum cholesterol by 13 mg/dl, and the addition of cholesterol to the diet increased serum cholesterol by an additional 12 mg/dl. The dietary cholesterol-induced change was principally in LDL cholesterol. A number of other minor changes in lipoprotein levels were also described, but the small number of subjects and variations in the experimental diets prevent extensive analysis. In these experiments, subjects with heterozygous FHC were not unusually sensitive to dietary cholesterol.

Uremic patients.--Green et al. (1985) fed six subjects with chronic renal failure (four on dialysis) for four weeks diets with 175, 425, and 875 mg cholesterol per day derived from eggs. No significant changes in serum cholesterol or triglycerides were observed.

Gallstones.--Lee et al. (1985b) compared the responses to dietary cholesterol of 12 subjects (six men, six women) with asymptomatic gallstones with those of seven healthy women. The patients with gallstones consumed diets containing 500, 750, and 1000 mg cholesterol per day for three-week periods. The six controls received diets containing 500 and 1000 mg cholesterol per day. The gallstone patients showed higher biliary saturation indices at all levels of cholesterol intake. Plasma cholesterol or lipoprotein cholesterol levels were not reported.

Diabetics.--Paisey et al. (1984) studied 503 Mexican Type II diabetic subjects between 38 and 60 years of age. Cholesterol intake was assessed by interviews of each subject. Ischemic heart disease and peripheral vascular disease were determined by history and physical examination. The survey was conducted with outpatients in a general medical clinic over a 6-month

period. Subjects with myocardial ischemia (undefined) and myocardial infarction had significantly higher cholesterol intakes than did those with angina or no cardiovascular disease. Differences in cholesterol intake were not related to peripheral vascular disease. Stepwise logistic regression analysis showed a significant coefficient for cardiac ischemia and peripheral vascular disease. The validity of this finding is difficult to evaluate because of self-selection among the subjects, the cross-sectional nature of disease assessment, which measured prevalence rather than incidence; and the many metabolic complications of diabetes.

Summary and conclusions.--Some investigators find an interaction between dietary cholesterol and type of fat, but others do not, and this remains an interesting but unproven possibility which requires further study. Although evidence is still fragmentary, sensitivity to dietary cholesterol does not seem to be altered greatly by a variety of metabolic abnormalities, including FHC.

IX. Relationship of Dietary Cholesterol to Atherosclerotic Disease

Puerto Rico Heart Health Program.--In this long term prospective epidemiologic survey (Garcia-Palmieri et al. 1980), the nutrient intakes of 8,218 urban and rural men, ages 25 to 64 years, were examined for their relationship to subsequent incidence of coronary heart disease. Nutrient intakes were assessed by 24-h dietary recall. The average cholesterol intake of rural men was 356 (297) mg/day; of urban men, 439 (318) mg/day. The average serum cholesterol concentration of rural men was 195 (39) mg/dl; of urban men, 205 (42) mg/dl. However, there were no significant differences in cholesterol intake among men who subsequently developed myocardial infarction, men who died from coronary heart disease, men who developed angina pectoris, and men who had no manifestations of coronary heart disease.

Western Electric Study.--Nearly 2,000 men, 40 to 55 years of age, were surveyed in 1957 and again 1958 for their dietary intakes by standardized interviews and questionnaires (Shekelle et al., 1981). The health status of these men was determined 20 years later and the predictive value of dietary intakes for death and for coronary heart disease calculated. The risk of death from coronary heart disease during the 19 year follow up was significantly associated with cholesterol intake measured at the initial interviews as mg/1000 Kcal. The logistic regression coefficient for dietary cholesterol, taking into account the known risk factors such as age, blood pressure, and smoking, was 0.003 (P=0.008). The intake of polyunsaturated fatty acids also was associated independently and inversely with risk of death from coronary heart disease, but saturated fatty acid intake was not.

Framingham Study.--Dawber et al. (1982) estimated the egg intake of 912 subjects in the Framingham Study from dietary histories taken in 1957. Egg

consumption varied from 0 to 24 per week in men (mean, 5.9/week) and 0 to 19 per week in women (mean, 3.8/week). There was no relationship of egg consumption to serum cholesterol concentrations, and no differences between tertiles of egg consumption in incidence of death from all causes, all types of coronary heart disease, myocardial infarction, or angina pectoris.

Combined studies.--Gordon et al. (1981) analyzed data on nutrient intake and incidence of coronary heart disease for up to 6 years from the Framingham Study (859 men), the Honolulu Heart Study (7,272 men), and the Puerto Rico Heart Health Program (8,218 men). The relationship was tested by comparing mean intakes of individuals who developed an event with intakes of those who did not, and also by computing logistic regression coefficients for the independent variables. Cholesterol intake averaged 528 (279) mg/day in Framingham, 414 (314) mg/day in Puerto Rico, and 549 (315) mg/day in Honolulu. No relationship of cholesterol intake to any type of coronary heart disease could be demonstrated.

Zutphen Study.--Kromhout and Coulander (1984) followed, for 10 years, 871 men whose dietary intakes had been assessed in 1960 by a cross-check dietary history method. There were slightly higher absolute and relative intakes of dietary cholesterol for the 30 men who died from coronary heart disease as compared with 857 who did not, but the difference was not significant.

Honolulu Heart Study.--This longitudinal epidemiologic study followed abut 7,000 men of Japanese ancestry, free of major chronic disease, for 10 years (McGee et al., 1984). Dietary intakes were assessed by 24-h recall at the initial examinations between 1965 and 1968. There were no significant differences between persons who developed coronary heart disease during the 10-year follow up and those who did not in age-adjusted intake of cholesterol expressed as mg/day. However, persons who developed any form of coronary

heart disease and those who experienced either myocardial infarction or coronary heart disease death had consumed significantly more cholesterol expressed as mg/1000 Kcal. A similar result was obtained by multivariate logistic regression analysis. Coefficients for cholesterol per 1000 Kcal were positive and were significantly different from zero for total coronary heart disease and myocardial infarction or coronary death.

Similar results were presented by McGee et al. (1985) from the same study in an analysis which included cancer and stroke as well as coronary heart disease. The coefficients for cholesterol intake and coronary heart disease were not compared to those presented in the previous report, perhaps because other variables were included in the regression analysis. Dietary cholesterol was not predictive of mortality from all causes or from cancer.

Ireland-Boston Heart Study.--Kushi et al. (1985) examined the relationship of dietary intakes with mortality from coronary heart disease 20 years later in 1,001 middle-aged men. Dietary intakes were assessed by a diet-history method between 1959 and 1965, and the vital status of the subjects was ascertained in 1982. Coronary heart disease deaths consumed an average of 266 (SE,7.5) mg cholesterol per 1000 Kcal, while those not dying of coronary heart disease consumed 248 (SE,2.7) mg/1000 Kcal ($P<0.03$). Logistic regression and proportional-hazards regression analyses yielded positive coefficients for dietary cholesterol and death from coronary heart disease, but of marginal significance ($P<0.10$).

Egg consumption and myocardial infarction.--Leitersdorf et al. (1986) compared 532 myocardial infarct survivors under age 65 years with 869 control cases with regard to a number of risk factors and other variables. They found no differences between cases and controls in history of egg consumption.

Summary and conclusions.--Three longitudinal epidemiologic studies (Western Electric Honolulu Heart, and Ireland-Boston Heart) found weak but statistically significant associations of dietary cholesterol intake with subsequent coronary heart disease, while three similar studies (Puerto Rico Heart Health, Framingham, and Zutphen) found none. Case-control studies were negative.

X. Reviews of the Diet-Heart Issue

Toward the end of the 1970's there appeared a number of review articles concerned with the diet-heart issue. Most of these dealt with the question of whether modification of dietary fat intake would reduce the incidence of atherosclerotic disease, and what recommendations should be made to the public. Few, if any, of these were focused specifically on the dietary cholesterol question.

Similar reviews continued to appear in 1980 and to the present. Ahrens (1979) addressed principally the reasons for not recommending a fat-modified diet for the general public, and did not discuss the effects of dietary cholesterol. Story and Kritchevsky (1980) discussed the many discrepancies and gaps in the evidence relating dietary lipids, including cholesterol, to atherosclerosis, and emphasized the diversity of opinion in interpreting the available evidence. James (1981) discussed the dangers in recommending diet modifications to the public before the likelihood of a beneficial effect was more firmly established.

Sinclair (1980) rejected the conventional "lipid hypothesis" and expressed the opinion that essential fatty acid deficiency might be more important. McMichael (1979) rejected the causal role of elevated serum lipids and emphasized the hazards of polyunsaturated fats in the diet, but did not discuss dietary cholesterol as an isolated factor.

Samuel et al. (1983) reviewed in some detail the aspects of cholesterol metabolism that are involved in the response to dietary cholesterol. They emphasized the variability of responses among individuals, and the difficulties involved in characterizing an individual's response as well as the metabolic basis for it. They suggested that an important research

objective was to develop rapid, inexpensive, and reliable methods of identifying these characteristics. The authors recommended that cholesterol intake be reduced in hyperlipidemic subjects, but did not believe that the evidence for a beneficial effect was sufficiently strong to recommend diet modification to the general public.

Stallones (1983) reviewed the epidemiologic evidence relating dietary and blood lipids to ischemic heart disease, but did not focus on dietary cholesterol. He emphasized the incomplete and conflicting epidemiologic evidence, and concluded that the relationship was not sufficiently well established to recommend dietary modification to the public.

Mitchell (1984,1985) rejected as myths the common beliefs that coronary heart disease is caused by atherosclerosis, that lipid deposition is important in atherosclerosis, that animal models simulate human atherosclerosis, and that serum cholesterol is determined by diet. He did not address dietary cholesterol specifically.

McNamara (1982) discussed several aspects of the diet-heart issue, including the limited responses of most subjects to dietary cholesterol, and the unknown frequency of responders. He concluded that recommendations for diet modification would be premature.

Vaupel and Graham (1980) calculated that totally eliminating eggs from the U.S. diet "might increase life expectancy by 20 days", and classified egg consumption as a "microrisk". The article was focused almost entirely on dietary cholesterol, and did not discuss fat intake. Although entertaining and instructive with regard to some aspects of public policy, such as comparing the effectiveness dietary modification with cessation of smoking and use of passive restraint devices in automobiles, it hardly represented a scientific analysis of the problem. Gruberg and Raymond (1981) championed the

etiologic role of vitamin B_6 deficiency in causing elevated blood homocysteine and atherosclerosis, and expressed the opinion that dietary fats were not important in causing atherosclerotic disease.

Mann (1979) responded to McMichael with a more positive assessment of the prudent diet, and recommended decreased total fat and increased polyunsaturated fat intake without mention of dietary cholesterol. Grundy (1980) reviewed briefly the major issues involved in the relationship of diet to coronary heart disease, including dietary cholesterol, and supported the recommendations that reduction in cholesterol intake, as well as other dietary fat modification, would be beneficial. Malmros (1980) reviewed the early history of the diet-heart issue, with particular emphasis on the observations of decreased mortality associated with altered food intake during World War II in northern Europe, but did not discuss the independent effects of dietary cholesterol. Connor (1980) reviewed his work on dietary cholesterol and emphasized the need to reduce cholesterol intake to reduce plasma cholesterol levels. Keys (1980) discussed broad aspects of the diet-heart issue in response to Werko (1979) but did not discuss the role of dietary cholesterol. Walker (1980) briefly mentioned the controversial nature of the effects of dietary cholesterol, and concluded by supporting recommendations to modify fat intake. Frantz (1981) supported the recommendations for reducing dietary cholesterol as well as reducing fat intakes.

Oliver (1982) reviewed in detail the epidemiologic evidence regarding diet and coronary heart disease, and concluded that dietary cholesterol had a minor role in determining plasma cholesterol concentrations because of the many compensating metabolic mechanisms. He estimated that a change in cholesterol intake from 250 to 750 mg/day would alter plasma cholesterol by less than 10 mg/dl. Overall, he concluded that the evidence relating dietary cholesterol to coronary heart disease was weak.

Connor and Connor (1983) reviewed intensively the effects of dietary cholesterol in humans and emphasized the evidence indicating a major effect. The authors attributed the discrepancy between metabolic ward and outpatient studies mainly to poor control of the experiments, lack of double-blind design, and the "ceiling effect"--the cholesterol intake above which no further change in plasma cholesterol occured. They concluded that dietary cholesterol was important in increasing plasma cholesterol concentrations, particularly LDL cholesterol concentrations, and in causing coronary heart disease.

Brown (1983), in reviewing diet and serum lipids, concluded that, with intakes below 300 mg/1000 Kcal, each 100 mg/1000 Kcal altered plasma cholesterol by 5 mg/dl. Grande, in the same symposium (1983), briefly reviewed dietary experiments in Europe and concluded that dietary cholesterol had a modest effect on plasma cholesterol.

Packard and Shepherd (1985) reviewed the pathophysiology of cholesterol metabolism in man and concluded that there was "a clear relationship between the amount of cholesterol in the diet and the level of that lipid in the circulation". On the basis of kinetic studies of cholesterol metabolism during cholesterol feeding, they indicated that dietary cholesterol increased LDL synthesis rather than reducing LDL catabolism, despite the suppression of cholesterol biosynthesis by dietary cholesterol.

XI. Recommendations of Committees

American Heart Association (AHA).--The AHA published its first recommendations regarding diet and atherosclerosis in 1957 (Page et al., 1957). Those recommendations included the reduction of dietary cholesterol. Subsequently, the AHA reviewed and re-stated these recommendations with additional supporting evidence and slight modifications, but continued to include reduction of dietary cholesterol and defined the upper limit at 300 mg/day. The most recent statement was issued in 1978 (AHA,1978), and it also included a limitation of cholesterol intake to 300 mg/day.

The AHA Nutrition Committee prepared a review of the AHA recommendations titled "Rationale of the Diet-Heart Statement of the American Heart Association" (Grundy et al., 1982) which reiterated the 1978 recommendations and presented an extensive review of the supporting evidence. The review acknowledged that there was much individual variation in response to dietary cholesterol, and estimated that each 100 mg/day decrease in dietary cholesterol resulted in an average decrease of 7 mg/dl of plasma cholesterol concentration. Reiser (1984) responded to this report in an editorial in which he reviewed the conclusions of each of the references cited regarding the role of dietary cholesterol, and pointed out that either the author's conclusions were more conservative about the effects of dietary cholesterol, or that the conditions of the experiment (extreme intakes of cholesterol or formula diets) made the results not applicable to recommendations for the public.

AHA diet recommendations for children.--In 1978, the AHA (Glueck et al., 1978) recommended that children with hypercholesterolemia be placed on a fat-modified die to reduce this risk fo adult coronary heart disease. The

recommendation included the reduction of dietary cholesterol to 200 mg/day, or less in younger children. With regard to the more controversial issue of diet modification for all children, it stated, "although the evidence does not yet support the recommendation that cholesterol and saturated fat should be reduced in the diet of all children, the public should be advised that such modification appears safe and very likely to be beneficial". This statement was widely regarded as too radical by some, and too conservative by others, but it was acceptable to the majority of the AHA at that time.

In 1983, the AHA (Weidman et al., 1983) issued a statement titled "Diet in the healthy child, which extended the recommendations for diet modification to all children over two years of age. This recommendation included a dietary cholesterol intake of "... approximtely 100 mgs. cholesterol per 1,000 calories, not to exceed 300 mgs."

National Heart Foundation of Australia.--This group recommended (1979), among other modifications of fat intake, "...restriction of cholesterol containing foods." The report acknowledged, in the background for the recommendation, "The effect of exogenous cholesterol differs from that of saturated fat in being smaller and more variable".

National Research Council, Food and Nutrition Board..--This brief report (1980) was concerned with nutritional modifications that might alleviate cardiovascular disease, hypertension, cancer, and diabetes. In the discussion of cardiovascular disease, it acknowledged that cholesterol intake could, among other nutrients, influence serum lipids "in metabolic wards under rigid dietary control". The report cited the "curvilinear relationship between dietary cholesterol intake and serum cholesterol concentration in man, as evidenced by a slope that decreases with increasing cholesterol intake...". It attributed this effect to "poor absorption of cholesterol at high levels,

plus feedback mechanisms that adjust biosynthesis to body needs," and noted the failure to find significant correlations between cholesterol intake and serum cholesterol concentration in free-living persons in the U.S. Consequently, the Board made no specific recommendations about dietary cholesterol for the healthy person. The Board did recommend reduction in total fat and food intake commensurate with caloric needs, and recommended reduction in salt intake.

It is not possible here to review all the rebuttals, editorials, letters, and other articles that this report evoked. Although the review of the dietary cholesterol issue cited only a few highly selected articles, the description of the relationship of dietary cholesterol to serum cholesterol was substantially similar to the conclusion reached in a 1979 review (McGill, 1979), except that the probability that there existed a number of very sensitive individuals was not explicitly acknowledged. However, the Board interpreted the effect as not sufficient to recommend limitation of cholesterol intake for the general public.

US Department of Agriculture (USDA).--The USDA, in cooperation with the U.S. Department of Health, Education, and Welfare, published a brochure (1980) giving dietary guidelines for Americans. This brochure, because its audience was the general public, contained no review of the scientific background, but stated "...reduction in our current intake of total fat, saturated fat, and cholesterol is sensible."

American Council on Science and Health.--A report published by this group (Carol, 1980) discussed the diet-heart issue and included a brief review of the evidence related to the effect of dietary cholesterol on blood cholesterol levels and on coronary heart disease. The report made no recommendations regarding specific nutrients for the healthy person, but emphasized variety in food selection.

American Medical Association (AMA).--The Council on Scientific Affairs of the AMA recommended (American Medical Association, 1983) that the optimal serum cholesterol concentration be defined as equal to or less than about 180 to 200 mg/dl. In treating elevated serum cholesterol levels, it recommended fat-modified diets containing less than 300 mg cholesterol per day. The Council did not address the issue of recommendations to the public.

Inter-Society Commission for Heart Disease Resources (ICHDR).--The Atherosclerosis Study Group of the ICHDR (Kannel, 1984) reviewed epidemiologic, clinical, and experimental evidence regarding the causes and possible prevention of atherosclerotic disease. Dietary cholesterol was mentioned, but was not intensively reviewed. The report recommended that all persons should consume less than 250 mg of cholesterol per day.

National Heart Lung and Blood Institute (NHLBI).--A Consensus Development Panel convened by NHLBI (NIH, 1985) recommended, "All Americans (except children younger than 2 years of age) should be advised to adopt a diet that...reduces daily cholesterol to 250 to 300 mg or less." The report did not discuss in detail the evidence supporting this recommendation concerning dietary cholesterol.

Summary and conclusions.--All of the committee and agency statements that appeared from 1979 to 1985, with two exceptions, recommended that dietary cholesterol be reduced to between 250 and 300 mg/day, or less. The special AHA statement regarding children extended this recommendation to all children as well as hyperlipidemic children. The National Research Council-Food and Nutrition Board report noted the same observations about dietary cholesterol in humans as did the other statements, but it did not recommend a change on the basis of those observations. All the reports but one stressed total and saturated fat intake as more important than dietary cholesterol.

XII. Summary and Conclusions

Cholesterol intake.--There has been little progress in methods of assessing cholesterol intake in free-living persons. A high degree of day-to-day variablity has been repeatedly demonstrated. Some studies have suggested that several days, perhaps as many as seven, of dietary intake records are necessary to estimate cholesterol intake accurately. U.S. men in the mid-1970's were ingesting, on the average, about 450 mg/day, and women, about 300 mg/day. Related to caloric intake, both were ingesting about 200 mg/1000 Kcal, one-third less than in previous years. A few persons consumed over 1000 mg/day. About one-third of dietary cholesterol was derived from eggs.

Correlation of cholesterol intake with serum lipids.--There has been little change in the results of cross-sectional surveys which examine the relationship of habitual intakes of cholesterol to serum or lipoprotein cholesterol concentrations. In at least one study, a small but statistically significant correlation of dietary cholesterol with LDL cholesterol was obtained, but other studies have been negative. No new analyses of ecological correlations have been reported. There are both theoretical and practical reasons for the lack of individual correlations even though a true relationship might exist. There seems to be little value in conducting further studies of this type. On the other hand, longitudinal studies, in which cholesterol intake is tested for its relationship to atherosclerotic disease years later, are more promising and at the same time are more convincing, whether the results are positive or negative.

Modifying egg consumption.--Two new studies reported no effects when free-living persons were asked to reduce or increase egg consumption, but three studies did show modest effects. Unless new dietary techniques or

products are being tested, there seems little need to repeat further studies of this type.

Cholesterol formula or controlled diets.--All studies in which formula diets are used, or those in which diets are prepared in a central kitchen laboratory, or those in which other measures taken to control dietary intake, show small but definite effects of dietary cholesterol on serum cholesterol. The average changes differ between experiments, and all experiments show considerable variation in individual responses, with many subjects being non-responsive and a few showing large responses.

Explanation for varying results.--When an average effect is small, a much larger sample is required to detect it reliably. All experiments with dietary cholesterol reviewed here involve small samples. Therefore, many studies will not find an effect and therefore represent a Type II error. The detection problem is complicated when a small effect is made up of many small or zero effects and a few large ones, because most statistical models that are used to test for significance assume an initial normal distribution and a shift in that distribution to another normal distribution with a different mean value. The measurement-error problem with dietary cholesterol affects cross-sectional individual correlations, but does not affect not well controlled dietary experiments. The measurement-error problem with serum lipids and lipoproteins (day-to-day variability) affects all experiments and surveys unless multiple samples are taken.

Reproducibility of responsiveness.--One research group has concentrated on the problem of whether responsiveness to dietary cholesterol is a consistent individual characteristic that is reproducible over months and years. Their findings suggest that responsiveness is reproducible and consistent in some persons, but is very difficult to establish because

response is highly variable in some persons and repeated tests are required. The currently available results suggest that about 10 to 20% of caucasians in an industrialized society are sensitive to dietary cholesterol, defined as a change of serum or LDL cholesterol by more than 30 mg/dl with a change in dietary cholesterol of 300 mg/1000 Kcal.

Metabolic basis of responsiveness.--Cholesterol absorption, as measured by current methods, does not explain the differences in responsiveness. The possibility that absorption is involved cannot be rejected because it cannot be measured accurately. Dietary cholesterol reduces LDL receptor activity, increases bile acid excretion, and decreases cholesterol biosynthesis, but there are not sufficient data to conclude that any or all of these processes are responsible for the differences in responsiveness among individuals.

Interaction with other nutrients.--This topic has not been studied in sufficient depth for conclusive results. Despite occasional suggestive results, there is no consistent evidence that there is an interaction of dietary cholesterol with type of fat in its effect on serum lipid and lipoprotein levels. Tests for other interactions have been negative. The effects of dietary cholesterol appear to be similar in a variety of subjects, including those with familial hypercholesterolemia.

Effects on lipoprotein profile.--The excess serum cholesterol induced in some persons by dietary cholesterol is carried mainly in an increased number of particles of normal LDL. No other subtle and possibly atherogenic changes in serum lipoproteins have been detected despite much speculation about the possibility.

Relationship to atherosclerotic disease.--Three new reports from longitudinal epidemiologic studies have shown a small but statistically significant predictive value of dietary cholesterol intake for subsequent

coronary heart disease, but three have not. These results are generally consistent with the results of observations regarding dietary cholesterol and serum lipids.

Summary of reviews and recommendations already in the literature.--Reviews and committee or agency reports are remarkably consistent in their assessment of the effects of dietary cholesterol on serum lipids and potentially on atherosclerotic disease: a small effect for most persons, an occasional large effect for a few persons. They diverge sharply, however, in their recommendations for advice to the apparently healthy general population. This divergence appears to represent a difference in the magnitude of anticipated benefit required before recommending a change in diet to the public, and not a difference in the scientific evidence or the reality of a true dietary cholesterol effect on atherosclerosis and its sequelae. When the debate becomes intense and scientists and physicians are forced into adversarial positions, the common ground of the factual basis of the debate becomes obscured.

Future research opportunities.--The major defect in our knowledge in this area is that detection of the cholesterol-sensitive individual is cumbersome and difficult, and we do not know the metabolic abnormality underlying this characteristic. The advances in molecular biology and molecular genetics of lipoprotein metabolism offer many attractive and testable hypotheses to explain this trait. Identification of the metabolic defect is likely to provide a marker by which the susceptible person can be identified. With such a resource, we could readily ascertain the frequency distributon of responsiveness. This information would not only be valuable to the public health physician in formulating recommendations to the public, but also would be useful to the clinical physician in the clinical medicine approach to the management of hyperlipidemia.

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APPENDIX B. SUMMARY OF THE PROCEEDINGS AND RESEARCH RECOMMENDATIONS FROM THE WORKSHOP ON THE IMPACT OF DIETARY CHOLESTEROL AND PLASMA LIPOPROTEINS AND ATHEROGENESIS

Abbreviations

Standard deviation	SD or ( )
Standard error	SE
Not significant	NS
Low density lipoprotein	LDL
Very low density lipoprotein	VLDL
Intermediate density lipoprotein	IDL
High density lipoprotein	HDL
3-hydroxy-3-methylglutaryl Coenzyme A	HMG-CoA
Familial hypercholesterolemia	FHC
Polyunsaturated to saturated fatty acid ratio	P/S

A Workshop on Dietary Cholesterol, held in Bethesda, Maryland, on July 1-3 1986, was cosponsored by the National Heart, Lung, and Blood Institute and the United States Department of Agriculture. Its purpose was to review existing data relating dietary cholesterol to risk for coronary heart disease (CHD) and to define future needs for research on this possible linkage. The workshop encompassed a broad range of topics ranging from basic science to epidemiology, and it reviewed research involving both humans and laboratory animals.

A possible relation between dietary cholesterol and atherosclerosis was first brought to light in laboratory animals. The feeding of cholesterol to many species--rabbits, chickens, and even primates--can induce a marked hypercholesterolemia and atherosclerosis. The multitude of research studies carried out in different kinds of laboratory animals has had a major influence on thinking about the role of dietary cholesterol in the genesis of CHD in humans. This is especially true, given the demonstration that many nonhuman primates are susceptible to the plasma cholesterol-raising effects of dietary cholesterol. However, for many years, skeptics have questioned whether findings in laboratory animals can be extended to humans. Clinical investigations have revealed that addition of excess cholesterol to the diets of humans does not induce a marked hypercholesterolemia, and for this reason, it has been proposed that man is basically resistant to dietary cholesterol. Thus, some workers contend that little or nothing is gained by way of reduction of coronary risk by restricting dietary cholesterol. The workshop participants examined the basis for these opposing views by considering the known or potential effects of dietary cholesterol from several different angles.

Dietary Cholesterol and Plasma Cholesterol

Several species of animals have been shown to be susceptible to diet-induced atherosclerosis; most notable are rabbits, pigeons, chickens, pigs and several species of nonhuman primates. All of these species except nonhuman primates are extremely sensitive to dietary cholesterol and develop severe hypercholesterolemia and atherosclerosis. In recent years, however, the primate models have gained the most popularity since they are phylogenetically closer to man and have similar lipoproteins to humans. Some nonhuman primates are "hyperresponsive" to dietary cholesterol and develop marked hypercholesterolemia on high intakes of cholesterol; others are more resistant to dietary cholesterol and raise their levels only to the range of 250 to 350 mg/dl. Thus, variability in response in plasma to dietary cholesterol is a characteristic of primates, and variability is even more pronounced across species. For example, in contrast to rabbits and chickens, rats and dogs respond to a high intake of dietary cholesterol with little or no rise in plasma cholesterol. Hence it is not possible to predict, on the basis of a study of laboratory animals, the response of humans to dietary cholesterol.

In general, however, humans are more resistant to dietary cholesterol than most nonhuman primates, in the sense that humans rarely develop striking hypercholesterolemia even when the intake of cholesterol is high. Still, the precise sensitivity of humans to dietary cholesterol is a matter of dispute. The results of several investigations seem compatible with the concept that dietary cholesterol has almost no effect on the plasma cholesterol. This conclusion has been drawn from a series of studies

carried out in outpatients in whom subjects were given ad libitum diets. On the other hand, in carefully controlled, metabolic ward investigations, increasing the cholesterol content of the diet has been shown to raise the plasma cholesterol. Such studies have been carried out in the laboratories of Keys, Hegsted, Mattson, Connor, and others. Although identical results have not been obtained in all of these studies, an overall pattern has emerged; most have shown that increasing the dietary cholesterol causes a rise in the plasma total cholesterol. When all data are taken together, the increase averages about 10 mg/dl for every 100 mg dietary cholesterol per 1000 calories. Thus, for a person who consumes 2000 calories per day, increasing the dietary cholesterol from 300 to 500 mg/day will cause an increase in the plasma cholesterol averaging 10 mg/dl.

One issue that was discussed is whether the increase in plasma cholesterol in response to an increment in dietary cholesterol is linear or curvilinear. This question remains unresolved for the circumstance in which cholesterol intakes are between zero and 500 mg/day; Hegsted et al and Mattson et al have reported a linear relationship, while Keys et al have claimed a curvilinear response. Within the usual range of cholesterol intakes, there is little difference in the absolute response whichever pertains. At higher intakes of cholesterol, i.e., greater than 500 mg/day, most workers agree that further increments in intake have little effect on the plasma cholesterol concentration. This may partly explain why giving additional cholesterol to the individuals on ad libitum diets fails to demonstrate a further rise in the plasma cholesterol. For practical purposes, the question is whether raising the cholesterol intake from about 250 to 500 mg/day will produce a significant rise in the plasma cholesterol.

According to the best available data from metabolic ward studies, increases in dietary cholesterol in this range should raise the plasma total cholesterol by an average of 10 to 15 mg/dl.

One feature of the response to dietary cholesterol in humans is its variability. Some individuals demonstrate a rather striking increment in plasma cholesterol when dietary cholesterol is increased--perhaps two-to-three-fold above the average, while others have little if any change. This variability in response to dietary cholesterol differs from that to saturated fatty acids in that the rise in cholesterol levels with the latter is fairly constant. Indeed, even for individuals, the response to dietary cholesterol appears to vary from time to time. Although there appear to be hyperresponders and hyporesponders among humans, this phenomenon is difficult to demonstrate in a group of individuals studied several times over an extended period of time. Thus, some people who on one occasion appear to be hyporesponders may, on another, be hyperresponders, and vice versa. For this reason, it is unlikely that a simple cholesterol-loading test can be devised to define a given individual's susceptibility to dietary cholesterol.

Another question that was discussed but not resolved was whether the response to dietary cholesterol depends on other nutrients in the diet. For instance, a greater rise in the plasma cholesterol may occur if the diet is rich in saturated fatty acids. This effect was reported by Schonfeld et al, but in the minds of most, it has not been adequately proven. If dietary cholesterol does not raise the plasma cholesterol when intakes of saturates are low, as when the diet is low in total fats or high in unsaturated fatty acids, this could have implications for dietary

recommendations; this is to say, a greater emphasis might be given to reducing intakes of saturated fatty acids than for decreasing dietary cholesterol. Still, the issue has not been settled, and a definitive investigation of the quantitative plasma response to dietary cholesterol in the presence of a variety of nutrients remains to be carried out.

In summary, carefully controlled studies conducted under metabolic ward conditions leave little doubt that increasing the dietary cholesterol will induce a rise in the plasma total cholesterol in most people. The failure to detect this effect in several outpatient investigations seemingly is the result of several factors that affect the power of a study to detect a change: (a) the limited response in plasma cholesterol to dietary cholesterol (approximately 10 mg/dl per 100 mg cholesterol per 1000 calories). (b) the inherent variability in response from one individual to another, and (c) the diminishing response at higher intakes of cholesterol. Exogenous factors determining the variability of response have not been determined, although amounts and types of fat in the diet could be one. In particular, the quantity of saturated fatty acids ingested may be an important modulating factor.

Dietary Cholesterol and Plasma Lipoproteins

The major effect of dietary cholesterol on the profile of plasma lipoproteins is to raise the low density lipoproteins (LDL). The mechanism of this action may be two-fold. First, dietary cholesterol may suppress the activity of LDL receptors. Brown and Goldstein have demonstrated that the synthesis of LDL receptors by cells depends on the cellular content of

cholesterol. When cellular cholesterol rises, the synthesis of LDL receptors is suppressed; conversely, when cellular cholesterol falls, the formation of receptors is stimulated. Recent work indicates that the liver is the major site of removal of LDL. Since the primary fate of dietary cholesterol is hepatic uptake of chylomicron remnants, it is reasonable to assume that an excess of dietary cholesterol will suppress the activity of hepatic LDL receptors. Studies by Spady and Dietschy in laboratory animals indicate that addition of cholesterol to the diet leads to suppression of receptor-mediated uptake of LDL by the liver.

A second mechanism whereby dietary cholesterol might raise the plasma level of LDL-cholesterol is by enhancing the secretion of cholesterol into plasma with lipoproteins. This mechanism was described by Nestel, who noted that feeding an excess of dietary cholesterol to humans leads to an apparent increase in the secretion of cholesterol-rich intermediate density lipoproteins (IDL). An enhanced secretion of cholesterol-rich lipoproteins during the feeding of excess cholesterol may account for the high molecular weight LDL noted by Rudel and coworkers during the feeding of cholesterol to nonhuman primates. The LDL particles in these animals become greatly enriched with cholesterol esters, and most of the excess esters consist of cholesterol oleate; the latter appear to be of hepatic origin. Cholesterol oleate is the product of intracellular acylcholesterol acyl transferase (ACAT), whereas the normally more abundant LDL-cholesterol linoleate is derived through the action of lecithin cholesterol acyl transferase (LCAT) in plasma. Despite the formation of high molecular weight LDL in nonhuman primates during cholesterol feeding, the same phenomenon could not be documented in humans. Rudel conducted studies with Schonfeld et al in

which excess cholesterol was fed to human subjects, and LDL of abnormally high molecular weight could not be identified.

Investigations of lipoprotein kinetics have been carried out in humans to determine the effects of dietary cholesterol on the metabolism of LDL. In one study, Ginsberg et al were unable to obtain a change in LDL concentrations by adding cholesterol to the diet. Measurement of turnover of LDL showed no change in production rates or fractional catabolic rates (FCRs) of LDL. Thus, when the plasma LDL level does change in response to cholesterol, apparently no change occurs in the turnover of this lipoprotein. In another study by Packard et al, LDL-cholesterol rose by 40% when cholesterol was fed, and this increment was associated with a decrease in FCR and an increase in production rate of LDL. In converse, Kesaniemi and Grundy reported that when absorption of cholesterol was inhibited by neomycin, the plasma level of LDL fell and the kinetic parameters were reversed, i.e., the production rate for LDL fell while the FCR increased. The changes in FCR for LDL in both of these latter investigations probably can be explained by alterations in receptor-mediated clearance of LDL. The changes in production rates of LDL are more difficult to explain. For example, the increase in production of LDL associated with the feeding of cholesterol was perhaps due to an enhanced influx of cholesterol-rich lipoproteins, but it was more likely due to a suppression of receptor-mediated removal of VLDL remnants, the precursors of LDL. VLDL remnants are thought to be removed by LDL receptors just as LDL itself.

Another lipoprotein that undoubtedly is affected by dietary cholesterol is the chylomicron. Almost all newly absorbed cholesterol enters the body

with chylomicrons. As the cholesterol content of the diet increases, so does the cholesterol content of chylomicrons. This means that the remnants of chylomicrons will be enriched in cholesterol. The spectrum of cholesterol-rich, post-prandial lipoproteins has not been thoroughly studied for humans. However, Mahley et al have isolated a form of cholesterol-rich particle in cholesterol-fed animals that appears to be a chylomicron remnant. It contains apo B-48 and apo E, and it is cleared from plasma by apo E receptors. The clearance of this lipoprotein by the apo E receptor is adequate under normal circumstances, and they accumulate in plasma only when there is an excess of cholesterol in the diet.

An excess of dietary cholesterol also may affect triglyceride-rich lipoproteins of endogenous origin. These lipoproteins likewise have been studied in dogs and swine by Mahley and coworkers. During the feeding of cholesterol to these animals, cholesterol-rich VLDL, called beta-VLDL, accumulate in plasma. Beta-VLDL seemingly are remnants of VLDL that have the beta-electrophoretic mobility of LDL, but in the ultracentrifuge, they float like triglyceride-rich lipoproteins. Beta-VLDL of this type contain apolipoprotein B-100, and they are removed from the circulation by VLDL receptors (B/E receptors). They accumulate in plasma because LDL receptor activity is downregulated by the increased dietary cholesterol, and as they circulate in plasma, they acquire increasing amounts of cholesterol. Beta-VLDL of cholesterol-fed animals resemble VLDL remnants of the same name that accumulate in humans in familial dysbetalipoproteinemia (type III hyperlipoproteinemia).

Cholesterol-ester rich VLDL might be expected to accumulate in the plasma of humans during the feeding of dietary cholesterol to humans. Scattered reports in the literature indicate that dietary cholesterol can increase VLDL-cholesterol as well as LDL-cholesterol. Furthermore, Nestel et al have reported that feeding of cholesterol to humans increases the secretion of cholesterol-rich VLDL and IDL.

The influence of dietary cholesterol on the metabolism of HDL is complex and poorly understood. An excess of cholesterol in the diet can actually raise the cholesterol content of HDL. A mechanism for this response may be related to the usual connection between HDL and triglyceride-rich lipoproteins. Normally, cholesterol esters of HDL are transferred to VLDL and chylomicrons and their remnants by cholesterol transfer proteins. This process involves the exchange of cholesterol esters for triglycerides. However, if chylomicrons and VLDL are already rich in cholesterol, because of an excess of cholesterol in the diet, the movement of cholesterol ester from HDL to triglyceride-rich lipoproteins may be blocked. As a result, cholesterol esters would become "trapped" in HDL. Normally, a high level of HDL-cholesterol should reflect a low concentration of triglyceride-rich lipoproteins, which should retard the exchange of cholesterol ester for triglycerides; but in an individual on a high-cholesterol diet, a high HDL-cholesterol may reflect the presence of triglyceride-rich lipoproteins that are enriched in cholesterol, so that transfer of cholesterol from HDL to VLDL or chylomicrons is inhibited.

Metabolism of Dietary Cholesterol

A critical issue pertaining to the significance of dietary cholesterol is that of cholesterol absorption. Numerous studies indicate that the absorption of dietary cholesterol is incomplete. Values for absorption have been reported to range from 25% to 75%, with considerable individual variation. Whether the absorption of cholesterol can be measured accurately in man has been the subject of considerable debate. Most methods require the use of radioactive tracers, and some workers question whether the rate of uptake of a tracer by the intestinal mucosa accurately reflects mass transfer across the intestine. Several investigators have claimed that luminal cholesterol exchanges with mucosal cholesterol, and if so, uptake of radioactivity would overestimate absorption. Other workers, notably McNamara and coworkers, have presented data that significant lumen-mucosa exchange does not occur, and hence uptake of radioactive cholesterol should faithfully reflect the mass absorption of dietary cholesterol. This is an issue that needs to be resolved with certainty so that accurate measurements of cholesterol absorption can be made with confidence.

One puzzling result from measurements of cholesterol absorption is that a constant fraction of cholesterol is absorbed regardless of the cholesterol intake. Since the absorption of cholesterol is limited, this finding is contrary to what might be expected. A decreasing percentage absorption of cholesterol with increasing intakes would be anticipated. On the other hand, the finding of a constant fractional absorption at variable intakes could be easily explained by exchange of radioactive cholesterol with mucosal cholesterol. Thus, the observation of apparent constant

fractional absorption over a broad range of cholesterol intakes suggests the need for further evaluation of the exchange question. Even if use of radioactive cholesterol as a tracer does accurately reflect fractional absorption, it may not always provide an integrated value of cholesterol absorption. The timing and frequency of administration of isotope relative to cholesterol intake must be taken into consideration.

Despite the methodological problems associated with the measurement of cholesterol absorption, abundant data indicate that absorption is variable from one person to another. Therefore, it can be asked whether the fractional absorption for an individual affects his or her plasma cholesterol level. McNamara indicated that he could find no relationship; in his studies, the percentage absorption of dietary cholesterol was measured in a sizable group of men, and no correlation was noted. Miettinen, in contrast, reported a different finding. He systematically divided a group of middle-aged Finnish men into three subgroups--those with high, moderate, and low levels of LDL. Percentage absorption of cholesterol was determined for all men in each of these groups; those with the highest LDL levels were found to have the highest percentage absorption of cholesterol, while those with the lowest levels of plasma LDL had a low cholesterol absorption. Therefore, Miettinen proposed that the percentage absorption of cholesterol does have a significant effect on the plasma cholesterol within a given population. Limited data from studies in different populations appear to support this latter concept. Some populations, such as American Indians, have both a low level of plasma cholesterol and a low absorption of dietary cholesterol. Nevertheless, because of the contrary data of McNamara, the issue of the relation between cholesterol absorption and plasma cholesterol is by no means resolved.

Still another question about effects of dietary cholesterol is whether the increment in absorption of cholesterol inhibits the synthesis of cholesterol. If feedback inhibition were to balance exactly the increment in absorption, the net change in body pools of cholesterol should be zero, and dietary cholesterol would be without clinical significance. However, the very fact that increasing the quantity of cholesterol in the diet raises the plasma cholesterol level in some people indicates that feedback control is not always perfect. McNamara has examined the variability of cholesterol synthesis in mononuclear cells from a series of patients loaded with an excess of dietary cholesterol. Most of the patients did not exhibit an increase in plasma cholesterol, and these patients apparently had adequate suppression of cholesterol synthesis. In contrast, about 20% of patients responded with a rise in plasma cholesterol levels, and these individuals failed to exhibit feedback inhibition. McNamara thus postulated that a subgroup of people have an insensitive feedback mechanism, and the individuals of this subgroup are those most likely to respond to dietary cholesterol with a rise in plasma cholesterol levels.

Finally, other compensatory mechanisms act to prevent the development of hypercholesterolemia during the feeding of large amounts of cholesterol to humans. For example, Grundy et al have demonstrated that an excess of newly absorbed cholesterol is partially resecreted into bile. This mechanism leads to an increase in biliary cholesterol during feeding of cholesterol, and it serves to rid the body of a portion of excess dietary cholesterol. Another protective mechanism theoretically could be an increased conversion of cholesterol into bile acids. This pathway serves to prevent hypercholesterolemia in cholesterol-fed rats and dogs. In humans, however, an

enhanced formation of bile acids in response to dietary cholesterol is inconstant, and does not appear to be an efficient means to avoid a rise in plasma cholesterol during the feeding of cholesterol to humans. The failure of enhanced catabolism of cholesterol to provide an effective pathway for elimination of newly absorbed, dietary cholesterol may be partially responsible for the rise in plasma cholesterol frequently seen during the administration of excess dietary cholesterol in humans.

Dietary Cholesterol and Risk for Coronary Heart Disease

Epidemiological evidence. Four published studies have shown an association of cardiovascular events with intakes of dietary cholesterol: the Western Electric, Zuthphen, Boston-Irish, and the Honolulu Heart Studies. All four investigations found a positive association between the intake of cholesterol and subsequent cardiovascular disease. Taken alone, however, these reports do not constitute adequate proof of a causal or clinically significant connection. It is important to remember the limitations of methods used in population-based studies for assessing the role of dietary cholesterol in particular, or for that matter, any nutrient.

Miscalculation of the true dietary intake tends to reduce the magnitude of any true association and may obscure it entirely. In most developed countries, for example, there is a substantial intraindividual variation in food intake from day to day, such that a 24-hour recall for one, two, or even three days may not be representative of the usual diet. The 24-hour recall does have the advantages of being more quantitative and less affected by forgetfulness, when it is compared with food frequency questionnaires.

For the assessment of dietary intakes, the best method probably combines food history, food frequency, and diet recall, but this approach is too cumbersome and expensive for use in large epidemiological studies; furthermore, it does not take into account changes in diet over time. Another problem in Western cultures is that the majority of the population may be eating a diet so high in cholesterol and saturated fatty acids that no low risk group is available for comparison. Finally, not only can there be miscalculation regarding dietary intakes, but also regarding the endpoint of greatest interest, atherosclerosis. Heart attack or sudden death may not be correlated perfectly with the extent of atherosclerosis (and hence with diet), because other factors (e.g., smoking, lack of exercise) may precipitate arrhythmia or coronary thrombosis--the proximate cause of a clinical cardiovascular event.

The four epidemiological studies showing a positive correlation between dietary cholesterol and development of CHD were conducted in middle-aged men, and with the exception of the Honolulu Heart Study, they were based on diet history rather than a 24-hour recall. Only in the Western Electric Study was the cardiovascular risk associated with dietary cholesterol shown to be independent of dietary saturated fat. Investigators in the Honolulu Heart Study felt that multicollinearity (e.g., persons eating high cholesterol diets also tend to eat high saturated fat diets) precluded meaningful multivariate analysis, while the issue of independence was not addressed in the Zuthphen or the Boston-Irish study.

The available observational studies are natural and potentially valuable experiments. Three types of studies -- comparison of different

populations, time trends in intake versus trends in mortality, and migration studies -- are all appropriate for evaluation of a common source epidemic. Investigators have always had great interest in unique populations, but it is difficult to draw definite conclusions because of the problem of confounding variables. For instance, populations with unique dietary habits usually differ from comparison populations in several other ways. Unique populations of interest are (a) one Nigerian group that consumes a diet high saturated fat and yet eats very little animal fat and cholesterol, and (b) ovovegetarians (compared with so-called pure vegetarians). As for vegetarian groups, there is a difference of opinion whether ovovegetarians have a higher plasma cholesterol than pure vegetarians.

Trend analysis studies are complicated by problems of confounding, i.e., changes in diet may coincide with changes in other behaviors. For example, during the period that the mortality from heart disease was declining in the United States, there were remarkable changes in cigarette smoking habits, blood pressure control, and treatment of myocardial infarction. Thus, it is difficult to know the quantitative contribution of each factor to the decline in overall cardiovascular mortality.

Among the migrant studies, the Ni-Hon-San Study of Japanese men is one of the best of its kind. In this study, the intake of dietary cholesterol, the plasma cholesterol, and mortality from heart disease increased in a gradient for men who remained in Japan, moved to Hawaii, or came to San Francisco. Many changes in lifestyles besides diet occurred in the different groups, however, complicating the interpretation of results.

Although a recent USDA survey of food consumption in the United States, based on a one-day food recall, included relatively small samples in a limited age range, the results suggest that the primary source of dietary cholesterol for U.S. adults is meat, poultry, and fish; milk is in second place, and eggs are a close third. A broad category, grain products, that includes mixtures in which animal products are ingredients, such as pastries or macaroni and cheese, ranks fourth. The use of eggs as the primary source of dietary cholesterol has apparently declined in the United States; at present only 15 to 18 percent of cholesterol intake in adults comes from eggs. In addition, eggs represent only four percent of total fat intake. Nonetheless, consumption of eggs was still the best dietary variable to distinguish adults in the lowest and highest categories of cholesterol intake. If these results are confirmed by larger surveys, the American diet may have changed enough to distinguish between the effect of dietary cholesterol and that of dietary fat on the plasma cholesterol. However, interpretation would again be confounded by other changes, such as the increase in dietary fiber intake since 1977.

If the cholesterol intake has declined in recent years, has this change caused a corresponding fall in plasma cholesterol? And if so, has this fall contributed to the decline in CHD mortality in the United states? There are problems in extrapolating between alteration in diet and changes in rates of heart disease. The American population has experienced a remarkable 40 percent decline in cardiovascular death rates that began around 1967. This decrement is too large to be explained by methodological phenomena such as changes in disease classification. Data from the Health and Nutrition Examination Survey studies beginning in 1960 and ending in 1980 showed a modest but probably significant (at the population attributable

risk level) decrements in serum cholesterol. Unfortunately, the latter change is complicated by changes in survivorship bias as well. These decreasing rates of heart disease rates have been found in blacks and whites, but they may diverge according to sex. Some of these differences await explanation, since they do not seem to parallel known changes in diet or plasma cholesterol.

Predicted CHD risk from plasma cholesterol changes. Another way to estimate the impact of dietary cholesterol on CHD is to extrapolate from the known relationship between plasma cholesterol and risk for CHD. Epidemiological studies within the American public indicate that for every rise of 1 mg/dl in the plasma cholesterol there is an approximate 1% increase in coronary risk. This relationship has been remarkably constant among several different surveys. Furthermore, in the recent Lipid Research Clinics (LRC) Coronary Primary Prevention Trial, the relationship was found to hold in reverse, i.e., for every drop of 1 mg/dl in the plasma cholesterol during the administration of cholestyramine, the rate of development of CHD fell by 1%. Of course, this connection represents an average change in a large population, and it does not necessarily hold for all individuals. Nonetheless, we might speculate from this correlation and the reported effects of dietary cholesterol about the impact of the latter on risk for CHD.

According to Mattson, a change in cholesterol intake of 100 mg/1000 calories will raise the plasma cholesterol by 10 mg/dl. If this value holds, what then would be the impact of reducting the cholesterol intake from 500 mg/day, which is typical of middle-aged American men, to the

commonly recommended 300 mg/day? For a 2000/day calorie intake, this change in cholesterol intake would produce a fall in plasma cholesterol of about 10 mg/dl. According to the relationship between plasma cholesterol levels and CHD, a reduction in cholesterol levels of 10 mg/dl should decrease coronary risk by about 10%. Apparently the plasma cholesterol of Americans has fallen 6 to 10 mg/dl over the past 20 years, and some have speculated that this fall is due in part to a decrease in intake of dietary cholesterol. Goldman et al recently postulated that the decline in plasma cholesterol among Americans has contributed significantly to the decline in CHD mortality. Thus, a reduction in dietary cholesterol could be one factor responsible for falling death rates from CHD. This possibility is speculation, but it is consistent with available data.

According to the equation of Keys et al, a decrement in plasma cholesterol resulting from a decline in cholesterol intake from 500 to 300 mg/day would be less than 10 mg/dl. This equation would estimate a reduction in the plasma cholesterol of only 5 to 6 mg/dl, which would indicate that a 200 mg/day decrease in cholesterol intake would reduce coronary risk by only about 5%. However, even this relatively small change is not trivial when it is applied to the whole of the population, and in view of the fact that CHD is a multifactoral disease. On the other hand, an estimated increase in risk of 5 to 10 percent from the higher intake of dietary cholesterol indicates that the latter is not the major dietary influence on CHD risk. For example, by use of the same Keys equation, it can be estimated that decreasing the dietary saturated fat from 17 to 10% of total calories would reduce coronary risk by 19%.

"Independent" effect of dietary cholesterol on coronary risk. There has been a persistent concern that dietary cholesterol may have an atherogenic action that is independent of its effect on the level of LDL in fasting persons. Zilversmit first suggested that post-prandial lipoproteins containing dietary cholesterol are atherogenic. More recently, with increasing evidence that remnants of triglyceride-rich lipoproteins are atherogenic, several investigators have continued to speculate about an independent effect of dietary cholesterol on atherogenesis. Previous studies were inconclusive because sufficient information was not available at the time the concept was initially set forth. Proper measurements were not made because of a general lack of knowledge of the lipoprotein system. At this juncture, however, we are not simply rediscovering the wheel when we propose to look for lipoprotein abnormalities in the post-prandial state. We currently know better what to look for, and we are beginning to obtain the analytical tools to make the critical measurements. There are now many specific ideas to test, making this a particularly good time to initiate intensive studies on the role of dietary cholesterol in the formation of atherogenic post-prandial lipoproteins. There are data to indicate that increasing the intake of dietary cholesterol will increase the cholesterol content of chylomicrons and hence chylomicron remnants. Thus, if remnants are atherogenic, the higher the intake of dietary cholesterol, the more cholesterol should be carried into the arterial wall with chylomicron remnants. Dietary cholesterol likewise may enhance the cholesterol content of VLDL remnants which could enhance their atherogenicity as well. Although this concept does not seem very radical at first glance, the clinical implications could be profound. For instance, positive results could lead to a change in the long-standing practice of making

measurements of plasma lipids only after individuals have fasted. It is possible that new information about post-prandial lipoproteins could explain a sizable fraction of the epidemiological linkage between dietary cholesterol and CHD.

Recommended Research

Based on the deliberations of the workshop and ideas emerging from the presentations, the chairpersons of each session formulated several recommendations for future research. It was decided that no single question was of overriding importance or could be answered definitively by one study. Instead, several important areas were identified that deserve more thorough investigation, and in fact, further research might contribute greatly to our understanding of the relationship between cholesterol in the diet and atherosclerosis (and coronary heart disease). Five specific areas were considered to be worthy of future research.

Effects of dietary cholesterol on fasting plasma lipoproteins.

Several carefully controlled studies in patients confined on metabolic wards or institutions have shown that addition of cholesterol to the diet will increase the plasma total cholesterol. Although there has been some variability among studies, there is little doubt that dietary cholesterol can increase the plasma cholesterol, and that an average response can be determined. However, the range of effects of dietary cholesterol on cholesterol concentrations in the different lipoprotein fractions--VLDL, IDL, LDL, and HDL--as they occur when the individual has been fasting, have not been well defined. Whether the increase in fasting plasma cholesterol

occurs exclusively in LDL, or is distributed among the lipoproteins, is not known with certainty. If an increase occurs in HDL-cholesterol, it would be of particular importance to know in which HDL fraction this increase occurs--whether HDL_3, HDL_2, or apo E-rich HDL. Finally, particular attention should be given to effects of dietary cholesterol on IDL, a lipoprotein that may have an unusually enhanced atherogenicity.

One characteristic of the action of dietary cholesterol is a high variability in response. This variability, which also occurs in experimental animals, is much greater than that in response to changes in the composition of dietary fat, for example. The average response for any given lipoprotein species thus fails to convey a picture of the true variability, and the range of response for each species needs to be defined better.

Metabolic ward studies are consistent in showing that dietary cholesterol frequently raises the plasma cholesterol, but this effect has been difficult to reproduce in free-living populations, most likely because of the difficulty in controlling all the variables that affect the levels of cholesterol. Therefore, if a study could be designed at a reasonable cost that would preclude a false negative result and could employ individuals living outside an institution, such a study might be valuable as a guide for public health recommendations. However, it must be emphasized that an inconclusive clinical trial is worse than no trial at all. Furthermore, the anticipated results of this type of trial are not deemed to be of such overriding importance as to put it high above other initiatives on the priority list. The reason is as follows. A definitely positive study would support public health recommendations that reduction of dietary

cholesterol is advisable; however a negative trial would not allow the opposite conclusion because of increasing evidence that post-prandial lipoproteins, induced by dietary cholesterol, but not present in the fasting state, also may be atherogenic.

Effects of dietary cholesterol on post-prandial lipoproteins. Dietary cholesterol differs from newly synthesized cholesterol in that the former has unique pathways. Dietary cholesterol enters the body through the intestine while most of the formation of new cholesterol occurs in the liver and peripheral tissues. Therefore, before cholesterol from the diet can enter body pools, it must be transported first through the plasma. This transport occurs mainly in chylomicrons and chylomicron remnants, although dietary cholesterol may stimulate the production of other types of lipoproteins by the intestine. Furthermore, during its transport with intestinal lipoproteins, cholesterol may be redistributed to other lipoprotein species. These post-prandial changes, resulting in the formation of "remnant" lipoproteins, create the potential for enhanced atherogenicity that does not exist in fasting plasma. Recent research shows that some remnant lipoproteins are selectively taken up by macrophages, which increases the likelihood that they are uniquely atherogenic.

Consequently, more investigation is needed on the nature of post-prandial lipoproteins. The levels of these lipoproteins should be quantified, and their composition--both lipid and apoprotein--should be determined. The individual variability in the post-prandial response should be defined. Techniques need to be developed to isolate these lipoproteins so that their interaction with cells can be described. The kinetics, dynamics, and

interactions of post-prandial lipoproteins should be determined. New techniques need to be developed for the rapid identification and quantification of post-prandial lipoproteins so that their prevalence in sizable populations can be described. The modifying effects of different types of fatty acids in the diet on the characteristics of these lipoproteins also should be examined.

Influence of dietary cholesterol on cholesterol metabolism in humans. Effects of dietary cholesterol on the metabolism of cholesterol in man are worthy of more research. Several processes need further investigation. These include the effects of cholesterol in the diet on (a) the absorption of cholesterol, (b) whole-body synthesis of cholesterol, (c) conversion of cholesterol to bile acids, (d) biliary secretion of cholesterol, (e) the activity of LDL receptors, (f) the accumulation of cholesterol in tissues, and (g) reverse cholesterol transport. Prolonged ingestion of dietary cholesterol may produce changes in cholesterol metabolism that are not apparent in short-term studies. These long-term effects of dietary cholesterol may not be reflected in fasting levels of plasma cholesterol. For example, an excess of dietary cholesterol may lead to the gradual accumulation of cholesterol in body pools, which could be atherogenic, without ever raising the plasma cholesterol level.

Since the response of plasma cholesterol to dietary cholesterol demonstrates considerable individual variability, the cause of this variability may reside in individual differences in response to dietary cholesterol as it affects one or more of the different parameters of cholesterol metabolism listed above. For example, an exaggerated rise in

plasma cholesterol might be the result of excessive absorption of dietary cholesterol; or alternatively, some patients may have an unusual rise in plasma cholesterol because of failure of feedback inhibition on cholesterol synthesis. Thus, the range of variability in response to dietary cholesterol on several parameters of cholesterol metabolism needs to be explored further.

Interactions of post-prandial lipoproteins and lipoprotein remnants with cells. Although the atherogenicity of LDL is well accepted, the role of remnants in atherogenesis is less well understood. Evidence of several types suggest that remnants contribute to the development of atherosclerosis. These remnants may be induced or enhanced by ingestion of dietary cholesterol. Several different types of remnants may be generated, and each needs to be investigated. These may be isolated from all of the lipoprotein fractions--chylomicrons, VLDL, IDL, LDL, and HDL. They could have several types of actions on cells that may be involved in the atherogenic process. These include cytotoxicity, interactions with different types of receptors, formation of foam cells, direct transfer of cholesterol from lipoproteins to cells, and interference with the release of cholesterol from cells. Many of these processes may be intimately involved in atherogenesis, and each requires further investigation. Different types of remnants may be involved in these processes, and therefore a study of remnant-cell interactions may open new avenues to a better understanding of the role of cholesterol-induced lipoproteins on atherogenesis and on the pathogenesis of atherosclerosis in general.

Effects of dietary cholesterol in experimental animals. Early studies in laboratory animals demonstrated that dietary cholesterol can induce hypercholesterolemia and thereby can cause atherosclerosis. However, many subsequent investigations that have taken advantage of this observation have been criticized because the amount of dietary cholesterol utilized to elicit the response is excessive and thus cannot necessarily be extrapolated to human beings. Nonetheless, with the appropriate selection of animal models and with the right experimental design, it should be possible to learn much about the effects of dietary cholesterol on metabolism of cholesterol and lipoproteins. An investigation of the types of remnant lipoproteins induced by dietary cholesterol would seem to be a particularly fruitful area for research, in particular studies in primates, some of which resemble humans in their response to cholesterol in the diet. Several unusual forms of cholesterol-induced lipoproteins already have been identified in laboratory animals, and some of these have subsequently been identified in humans. Although amounts of these lipoproteins differ, several lipoproteins are qualitatively similar among the species. Thus, more investigation in laboratory animals can be justified for the identification and characterization of post-prandial lipoproteins. Experimental animals can be used for other studies such as (a) the fate of dietary cholesterol, (b) effects of cholesterol in the diet on metabolism of HDL and reverse cholesterol transport, (c) influence of dietary cholesterol on metabolism of cholesterol, including transfer of cholesterol between tissues and lipoproteins, and (d) mechanisms of atherogenesis in response to cholesterol-induced lipoproteins.

APPENDIX C. ANALYSIS OF FEDERAL SUPPORT OF DIETARY CHOLESTEROL RESEARCH FOR FISCAL YEARS 1984-1985

APPENDIX C. ANALYSIS OF FEDERAL SUPPORT OF DIETARY CHOLESTEROL RESEARCH FOR FISCAL YEARS 1984-1985

Analysis of Federal Support of Dietary Cholesterol Research for Fiscal Years 1984-1985

Introduction

Federal support for dietary cholesterol research has resulted in much of the present knowledge about dietary cholesterol and its relationship to plasma cholesterol and coronary heart disease. Current research supported by Federal agencies is briefly reviewed below.

The Human Nutrition Research and Information Management (HNRIM) classification is used by Federal agencies to document and categorize support of nutrition research. The HNRIM data base was developed by the joint DHHS-USDA Task Force on the Human Nutrition Research Information and Management System in accordance with a Congressional mandate. The Task Force operates out of the Office of the Nutrition Coordinating Committee of the NIH under the guidance of an interagency committee cochaired by the Assistant Secretary for Health, DHHS and the Assistant Secretary for Science and Education, USDA.

Each participating agency (at present, the Department of Health and Human Services, Department of Agriculture, Veterans Administration, Agency for International Development, Department of Defense, and National Marine Fisheries Service of the Department of Commerce) assembles and submits its own data. Data from the participating agencies are combined into the central HNRIM data base.

For the purposes of this review, the HNRIM category of all FY 1984 and 1985 project descriptions in which the term "cholesterol" occurred in the title or narrative were initially considered. All project narratives were then reviewed in order to include only those pertaining to dietary cholesterol. It should be recognized that other projects, while not including dietary cholesterol in the title or narrative, include research related to dietary cholesterol. For example, studies concerned with dietary fat often relate to dietary cholesterol since the sources of dietary saturated fat and dietary cholesterol are often the same. Furthermore, dietary cholesterol is intricately associated with the lipoproteins, their concentrations, composition and metabolism. Thus a study primarily concerned with lipoprotein metabolism usually considers dietary cholesterol. For these reasons, this review probably underestimates the amount and scope of the research on dietary cholesterol being supported by Federal agencies.

This review includes only those studies in which dietary cholesterol is a major focus of the research. Not included are projects in which dietary cholesterol is an interwoven component of the research such as epidemiologic population based studies, methodologic studies to improve the measurement of dietary intake, food composition research or demonstration and education projects. Although some projects could appear under more than one heading, no project appears more than once.

A key to the identification codes for the various projects is provided at the end of this section on pages 20-21. The beginning letter and first two numbers of the code indicate the type of activity; the second two letters indicate the agency supporting the activity; the last numbers are the project identification numbers within the sponsoring agency.

A. Research Involving Humans:

1. Effects of dietary cholesterol on the levels of serum cholesterol and other lipoproteins

B10 UR 0095679 McNamara, D.J.
Plasma Lipids in Subjects with Cardiovascular Disease

This study involves the analysis of total and lipoprotein cholesterol and triglyceride concentrations of apolipoproteins B, A-1 and E in human subjects with cardiovascular disease before and after initiation of lipid lowering diets.

R01 HO 18593 Davis, Mayadee A.
Infant Milk Feeding and Serum Cholesterol: U.S. Children

It has been hypothesized that the high cholesterol content of breast milk sets up homeostatic mechanisms that allow for effective cholesterol metabolism in adult life. Commercial formulas (low in cholesterol) are concurrently the predominant infant milk feeding in the U.S. This study examines the relationship between the type of infant milk feeding and serum cholesterol levels of U.S. children aged 4-11. Multivariate techniques are being used to examine the effects of current age, sex, race, parental income, parental education, current body weight, current diet and age of introduction of solid foods on the relationship between type and duration of infant milk feeding and childhood serum cholesterol levels.

Z01 VA 47959 Anderson, James W.
Plant Fiber Effects on Glucose and Lipid Metabolism

This study is assessing the effect of feeding a diet containing 450 mg of cholesterol with 20 g of plant fiber/day on the lipoprotein and cholesterol levels in patients with hypercholesterolemia. Subsequently, the patients will be randomized to either an oat bran- or bean-supplemented diet and the measurements repeated.

Z01 UA 0047755 Berlin, E.

Effects of Dietary Lipid Modification of Plasma Lipoprotein Physical-chemical Properties

The objective of this study is to determine in humans and animals the effects of saturated fat and cholesterol on the physical and chemical properties of plasma lipoproteins, including particle size and those perturbations of metabolism that modulate lipoprotein-cellular receptor interactions.

T32 HL 07050 Longmore, William J.
Special Interest area: Multi-disciplinary Heart and Vascular Diseases

The study of the effects of dietary cholesterol on serum lipids and lipoproteins in humans is the main nutritional component of this project.

R01 HL 32000 Schonfeld, Gustav
Diet Effects on Lipoproteins of APO E_1 Heterozygotes

Plasma lipoproteins are characterized in subjects while they are eating habitual, basal and high fat and cholesterol diets. It is intended to correlate hypo- and hyper-responders to these diets with their APO E phenotype. It may then be possible to identify a population segment more prone to dietary-induced atherosclerosis.

R01 HL 20910 Connor, William E.
The Alternative Diet for Coronary Disease Prevention

The purpose of this study is to determine the effectiveness and acceptance of an "alternative diet" by a random sample of 233 families. The diet is low in cholesterol, saturated fat, total fat and sodium. Plasma lipids and lipoproteins are being determined. Dietary histories and evaluation of the study population to determine acceptance, and adherence to the diet, are being undertaken. Unique aspects are the focus on a family unit, and a gradual non-coercive and positive motivational approach.

2. Influence of dietary cholesterol on cholesterol and lipoprotein metabolism. This will include absorption of cholesterol, whole body synthesis, conversion to bile acids, biliary secretions of cholesterol, activity of LDL receptors, accumulation of cholesterol in humans, reversed cholesterol transport, etc.

Z01 UA 0047268 Behall, K.M.
Effect of Dietary Fiber on Metabolic Parameters in Humans

Z01 UA 0043757 Canfield, W.
Effects of Plant Fibers, Sucrose and Starch on Lipids and Carbohydrates

These studies measure the effects of dietary fibers on the absorption of dietary cholesterol and, in turn, their effects on serum lipids including serum cholesterol in human subjects.

R01 HL 23077 Ginsberg, Henry N.
Regulation of Apoprotein-B Metabolism in Humans

The effect of dietary and drug regimens that alter plasma cholesterol and triglyceride levels and the synthesis of apoprotein B and its degradation is being studied in individuals with normal and elevated lipid levels.

R01 HL 29074 Illingworth, D. Roger
R01 HL 32271
Lipid Metabolism and Therapy of Hypercholesterolemia

Steady-state metabolic studies are being conducted in patients with familial hypercholesterolemia and combined familial hyperlipidemia during sequential therapy with (a) a low cholesterol, low fat diet, (b) diet plus colestipol, (c) diet plus nicotinic acid, and in some cases (d) diet plus colestipol and nicotinic acid and (e) diet and mevinolin. These studies examine low density lipoprotein turnover, whole body cholesterol synthesis and LDL receptor activity, and compare those processes with lipoprotein concentration and composition.

B10 UR 0089302 Story. J.A.
B10 UR 0089303 Garcia, P.A.
B10 UR 0089370 Carrol, S.L.
B10 UR 0089519 Kies, C.V.
B10 UR 0089559 Kummerow, F.A.
Modification of Human Diets Designed to Affect Lipid Metabolism

The objective of the above studies is to compare, in individuals with normal lipid levels, the effects of dietary fat intake comparable to that reported in the USDA 1977 Food Intake Survey with the effects of a lower dietary fat intake recommended in Dietary Goals for the United States. Lipid and lipoprotein levels as well as cholesterol and lipoprotein metabolism are studied. In animal models the impact of the two dietary patterns will be determined on metabolic parameters not easily measured in humans.

B10 UR 0082319 Chen, S.C.
Dietary Pattern Interactions in Lipid Metabolism

The objective of this study is to assess the effects of different levels and types of dietary fat as well as low versus high levels of dietary cholesterol on plasma high density lipoprotein composition, concentration and metabolism. The roles of individual fatty acids or combinations of fatty acids in cholesterol, lipoprotein and prostaglandin metabolism will be defined. Included in the objectives is a desire to characterize optimal levels of the major fatty acid categories.

R01 HL 32435 Breslow, J.L.
Human Dietary Cholesterol Tolerance

The goal of this project is to evaluate the role of apoprotein genetic variations and metabolism in determining an individual's atherosclerotic susceptibility and response to saturated fat and cholesterol in the diet.

Clinical studies in humans are contemplated to examine the effect of dietary cholesterol and fat on apo E synthesis and catabolism and to determine whether DNA polymorphism associated with the apoprotein genes predicts atherosclerosis susceptibility and/or diet response.

P50 HL 21006 Goodman, DeWitt S.
Lipids, Lipoproteins, Platelets and the Arterial Wall

As part of the parent Specialized Centers of Research (SCOR) grant a number of projects are concerned with cholesterol turnover and metabolism in normal and hyperlipidemic humans.

T32 HL 07311 Holman, Ralph
Postdoctoral Training in Lipid Research

The role of high dietary intake of cholesterol and saturated fat in atherosclerosis is investigated as part of the training program of postdoctoral students in the general area of lipid biochemistry and its application to coronary artery and related diseases.

PO1 CA 2950207 Ahrens, E.H. as part of the Clinical Nutrition Research Unit of the Memorial Hospital for Cancer and Allied Diseases.

Included in the studies of cholesterol homeostasis is the measurement of cholesterol absorption and synthesis in patients on high versus low cholesterol diets that are either high in saturated fatty acids or high in unsaturated fatty acids.

KO1 HL 28727 McNamara, Donald J.
KO1 HL 35417
Nutrition and Cholesterol Homeostasis

These projects are designed to study nutritional effects on cholesterol homeostasis in free-living outpatients. The following are studied. (1) The effect of fat quality and cholesterol quantity on variations in the feedback control of cholesterol metabolism. (2) The effect of long-term ingestion of polyunsaturated fat diet on the size of the body pools of cholesterol. (3) The effect of early post-natal nutrition and cholesterol homeostasis. This study seeks to identify those people whose cholesterol homeostasis is affected by dietary factors in order to determine the effectiveness of dietary control in reducing tissue cholesterol concentrations, to define the effect of nutritional factors in cholesterol homeostasis and eventually to identify parameters that could characterize those persons whose control of cholesterol homeostasis is affected by dietary factors.

3. Dietary cholesterol and cancer

RO1 HL 32338 Hulley, Stephen
Serum Fatty Acids and Incidence of Coronary Heart Disease

The objective of this study is to provide new evidence on the nature of the relationship between habitual dietary fat and cholesterol intake and the development of coronary heart disease, and to examine the possibility that the association between low serum cholesterol levels and mortality from cancer or stroke is related to habitual dietary fat composition. These data will provide information on how the adoption of a fat-controlled diet by Americans would influence their commonest causes of mortality: CHD, cancer and stroke.

R23 CA 36008 Schultz, Terry D.
Diet Hormone Metabolism and Breast Cancer Risks

This study proposes to assess in vegetarian versus nonvegetarian women the interrelationships of major nutrients, including dietary cholesterol, with the various steroid and polypeptide hormones that are possibly related to the risk of developing breast cancer.

RO1 CA 26560 Speizer, Frank E.
RO1 CA 37088
RO1 CA 40356
RO1 CA 40935
Epidemiology of Diet and Cancer in Women

A study of diet and cancer is being conducted among a cohort of U.S. female registered nurses. A variety of hypotheses are being tested relating dietary factors, including cholesterol, to the incidence of cancer of the breast, colon and lung and malignant melanoma.

RO1 CA 29723 MacMahon, Brian
An Epidemiological Study of Renal Adenocarcinoma

One of the objectives of this study is to test the hypothesis that high dietary cholesterol increases risk of renal cancer. The study will compare data for 200 newly diagnosed patients and 125 previously diagnosed patients with renal cancer with healthy adults of the same sex, age and race residing in the same neighborhood.

RO1 CA 30983 Shekelle, Richard B.
Diet, Alcohol, Tobacco and Risk of Cancer in Men

This research is designed to explore the association between dietary variables (e.g., vitamins, animal fat, dietary cholesterol), alcohol consumption, use of tobacco and risk of malignant neoplasms in over 2000 middle-aged employed men who participated in the 1957-58 Western Electric Health Study.

RO1 CA 33619 Kolonel, Lawrence N.
Epidemiologic Studies of Diet and Cancer in Hawaii

This is an etiologic study, in the multi-ethnic population of Hawaii, of cancer in relation to dietary exposures to specific foods and their nutrient components such as fats, cholesterol and vitamins.

4. Methodology with particular reference to pooled information and its availability, methodology of dietary surveys and education programs, etc.

N01 HO 59001 Sardinas, Tony
Support for National Cholesterol Education Program

Based on the growing consensus that the reduction of elevated cholesterol can lead to a reduction in heart attacks, fatal and nonfatal, a program has been designed to educate health professionals and the public alike to take action and reduce elevated cholesterol levels. The program is based on scientific findings and the best collective judgment regarding implications of those findings, and involves numerous agencies and organizations in both the private and public sectors.

B. Research Involving Animals:

1. Effects of dietary cholesterol on the levels of serum cholesterol and other lipoproteins.

B10 UR 0078080 Strength, D.R.
Relationship of Diet to Cholesterol Concentrations, Pool Size and Turnover in Tissues of Rats

In this study ^{3}H-cholesterol is being used to measure cholesterol absorption and turnover by input-output rate of labeling cholesterol and its metabolites in rats.

B10 UR 0085280 Hegsted, M.
The Effect of Dietary Saponins and Cholesterol on Cholesterol Metabolism in the Rat

The effects of varying levels of purified saponins and of a diet with or without added cholesterol on cholesterol metabolism in the rat are being examined. Blood samples are analyzed for cholesterol and triglyceride levels; liver samples are analyzed for total lipid, cholesterol and phospholipid levels.

P30 AM 30865C2 Feldman, Elaine B.
Clinical Nutrition Research Unit

One of the projects entails the study in Rhesus monkeys of plasma lipoprotein and cholesterol response to varying levels of dietary cholesterol and fat.

R23 HL 28868 Kris Etherton, Penny M.
Age, Sucrose Diet and Lipoprotein Metabolism

The objective of this project is to examine aberration in the plasma lipoprotein profile, hepatic lipoprotein production, skeletal muscle and adipose tissue lipoprotein lipase and vessel and tissue pathology as influenced by age, exercise and diet-induced hyperlipemia.

R01 HL 24736 Rudel, Lawrence L.
Nutritional Effects on Nonhuman Primate HDL

The influence of dietary cholesterol, ethanol, lecithin, and unsaturated versus saturated fat on the plasma lipoprotein and lipid distributions and concentrations are being studied in African Green monkeys.

F32 HL 06700 Kammerer, Candace M.
Genetics of Atherosclerosis

Populations of baboons have been developed in which serum cholesterol levels increase or are unchanged when chow is changed to an atherogenic diet. Serum lipids, lipoproteins, and body weights are studied in parents and progeny and subjected to genetic analysis. The study focuses on the genetic control of serum cholesterol as well as environmental factors that may be involved.

P50 HL 14164 Clarkson, Thomas B.
Specialized Centers of Research-Atherosclerosis (SCOR-A)
Comparative and Experimental Athersclerosis

As part of the parent SCOR Program, particular emphasis is placed on the study of the genetic and biochemical mechanisms determining hypo- or hyper-responsiveness of nonhuman primates to dietary cholesterol, including changes in the structure and function of lipoproteins.

P60 HL 15103 Berenson, Gerald S.
Research & Demonstration Center Early Natural History of Atherosclerosis

This grant includes nutritional studies on non-human primates to develop models of hypertension and hyperlipoproteinemia: a high sucrose diet has resulted in an exaggeration of the effect of dietary cholesterol on serum lipoproteins and on self-induced hypertension.

2. Influence of dietary cholesterol on cholesterol and lipoprotein metabolism

B10 UR 0084582 Chen, S.C.
Effect of Premature Weaning on Cholesterol Metabolism in Later Life

Rats will be weaned prematurely at 16 days or normally at 30 days to a laboratory diet of known composition, but lower in fat and cholesterol. At 2, 11, and 23 months they will be challenged with a diet high in fat and cholesterol. Biochemical responses to this challenge will be measured, including several liver enzymes involved in cholesterol metabolism and serum cholesterol and lipoproteins.

B10 UR 0082177 Ostwald, R.
Dietary Cholesterol and Saturation of Fat. Plasma Lipoprotein, Cell Functions, Cell Survival

Guinea pigs, fed diets containing fats with different degrees of unsaturation, with or without cholesterol, will be used to determine (a) the apoprotein composition of individual plasma lipoprotein classes, (b) survival time in vivo of red blood cells enriched with cholesterol, and (c) the phagocytic activity of cholesterol-enriched macrophages.

RO1 AM 35174 Singhal, Anil K.
Role of Dietary Variations on Gallstone Formation.

RO1 AM 35375 Hayes Kenneth C.
Dietary Fats and Gallstones

These studies determine the effects of dietary constituents including cholesterol on various aspects of cholesterol/bile acid metabolism, composition of lipoproteins, interaction of plasma lipoproteins with hepatic membrane receptors, lipoprotein turnover and on lithogenic bile production. Hamster gallstone models are being used.

B10 UR 0088523 Maxwell, C.V.
The Influence of Dietary Factors upon Cholesterolemia in Two Animal Models

Cannulation of the bile duct and ileum is used to quantitate the effect of diet on the absorption, transport, and metabolism of cholesterol in a strain of atherosclerosis-susceptible turkeys and in the pig model. The investigator will also evaluate the effect of dietary cholesterol, fed as poultry eggs, on serum lipids and subsequent atherosclerotic plaque formation.

B10 UR 0095806 Beitz, D.C.
Animal Products and Human Health

This study evaluates diet composition on cholesterol homeostasis in the pig. Techniques are being developed for removing cholesterol from animal products and subsequently determine their effects.

B10 UR 0091960 Ott, D.B.
Biochemical Regulation of Cholesterol Metabolism

The objective of this study is to determine the role of dietary cholesterol on 2, 3-oxidosqualene sterol cyclase activity. The latter is involved in the enzymatic control of liver cholesterol biosynthesis. The animal model is the rat.

B10 UR 008231 Nishida, T.
Dietary Lipids and Plasma Lipoproteins

The metabolism of high density lipoproteins and low density lipoproteins as influenced by dietary cholesterol and fats is being studied in relation to atherosclerosis in experimental animals fed various diets differing in the amount and type of fats, cholesterol and other constituents.

R01 UG 0034040 Story J.A.
Mechanisms of Alteration of Bile Acid Excretion by Dietary Fiber

One of the objectives of this work is to examine the hypothesis that adsorption of bile acids by dietary fiber results in an increase of sterol excretion of sufficient magnitude to alter serum and liver cholesterol levels. Rats are fed semipurified diets containing fiber with or without 0.25% cholesterol.

Z01 UA 0046717 Chang, M.L.W.
Effect of Dietary Fiber and Lipid Metabolism and the Rate of Glucose Absorption

This study assesses the influence of dietary cholesterol fed with or in the absence of fiber on lipid metabolism and glucose absorption in rats.

R01 AM 27706 Field, F. Jeffrey
Regulation of Intestinal ACAT

The purpose of this project is to study the regulation of intestinal acylCoA:cholesterol acyltransferase (ACAT) by plasma lipoproteins and by phosphorylation-dephosphorylation. Twenty pairs of rats are surgically joined to form a parakontic union. One rat in a joined pair is fed a diet containing cholesterol (Diet A) and its mate a diet devoid of cholesterol (Diet B). In another pair one animal is fed diet B and one diet A (control diet). Microsomal ACAT and HMB CoA reductase activity is determined in the small intestine. Serum and microsomal cholesterol levels are also being measured. Conclusions will be made regarding the capability of the intestine in taking up and degrading lipoproteins as a source of cholesterol.

R01 AM 21367 Glickman, Robert N.
Chylomicrons-Formation, Structure and Function

The effects of dietary saturated and unsaturated lipids, and dietary cholesterol on intestinal apoprotein content and synthesis is being determined directly on rat intestinal mucosa and in human intestinal biopsies. These studies are providing information concerning factors which modulate the intestinal contribution to lipoprotein metabolism.

K08 AM 01221 Spady, David K.
Regulation of Hepatic LDL Transport in Vivo

Hamsters and rats are subjected to a variety of perturbations including dietary factors which are known to alter LDL concentrations. The studies help to define the relationship among rates of LDL uptake, rates of sterol synthesis and size of cholesterol ester pool in the maintenance of cholesterol homeostasis and on the mechanisms by which LDL concentrations are altered in several clinically important situations including cholesterol feeding.

K08 AM 01256 Magun, Arthur M.
The Intestine and Nascent Lipoproteins

The factors that influence the synthesis of the nascent lipoproteins by the intestine are studied by cholesterol and fat feeding, bile ligation and induction of a hypocholesterolemic state. The metabolism and catabolic fate of the lipoproteins synthesized by the intestine is being examined.

P01 AM 19329 Combes, Burton
Program Project in Liver Diseases

A part of this project is devoted to comprehensive studies in several species of cholesterol influx into the liver. This is done by infusing various classes of lipoproteins intravenously or by varying the rates of hepatic cholesterol synthesis by feeding cholesterol, infusing chylomicrons or by inhibiting cholesterol absorption.

R01 AM 13324 Holten, Darold D.
Nutritional and Hormonal Control of G-6-P04 Dehydrogenase

Within this project studies have been initiated to determine if the induction of apolipoprotein E by dietary cholesterol in rabbits proves a good system for studying the mechanism by which dietary cholesterol regulates protein systems in the liver.

T32 HL 7115 St. Clair, Richard M.
Special Interest Area: Cardiovascular Pathology

The nutrition component of this project is directed toward cholesterol lipoprotein metabolism in non-human primates that are hypo- and hyper-responsive to dietary cholesterol, and the effects of diet induced hypercholesterolemia.

R01 HL 32435 Breslow, J. L.
Dietary Cholesterol Tolerance

This nutritional study in monkeys is intended to increase the understanding of dietary effects on apoprotein synthesis in nonhuman primates and will obtain correlations between parameters of diet responsiveness and/or atherosclerotic tendency and apoprotein synthetic rates.

R01 HL 23525 Swift, Larry L.
Liver and Intestinal Golgi Lipoproteins

The cholesterol-fed rat is being used to investigate lipoprotein synthesis in the hypercholesterolemic state in both the liver and intestine.

R01 HL 23792 Nicolosi, Robert I.
Dietary Control of High Density Lipoproteins

This study assesses the dietary factors that influence HDL metabolism in two monkey models with contrasting levels of HDL subclasses. Various aspects of HDL synthesis and HDL uptake by the liver are correlated with species and diet induced variations of HDL subclass levels.

P01 HL 28972 McGill, Henry C. Jr.
Diet, Stress and Genotype in Primate Atherosclerosis

This research program studies in the baboon the roles of diet, genetics and environmental stress in the etiology and pathogenesis of atherosclerosis. One project will study interactions between dietary cholesterol and genetic dyslipoproteinemias. Another project completes a long-term experiment in which pedigreed baboons are reared to adulthood on different cholesterol and fat enriched diets to determine effects on cholesterol absorption and turnover, serum lipoproteins and atherosclerosis.

R01 HL 09610 Dietschy, John M.
Control of Cholesterol Metabolism-Bile Acid Transport

Part of this project involves purification of solubilized HMG CoA reductase and an attempt to develop a specific antibody for this rate-limiting enzyme of cholesterol synthesis. Such an antibody, in conjunction with appropriate kinetic studies, is used to examine physiologic maneuvers known to affect cholesterol synthesis such as cholesterol feeding, stress, etc.

R01 HL 09744 Portman, Oscar W.
Experimental Vascular Sclerosis

The aims of this research are to measure the effects of dietary factors--fat, cholesterol and protein--and of gender and sex hormones on lipoprotein composition and metabolism, and on bile composition and metabolism in two species of monkeys.

R01 HL 10933, R01 HL 10940 Zilversmit, Donald B.
Fat Metabolism and Experimental Atherosclerosis

This research addresses the role of triglyceride-containing lipoproteins in experimentally produced atherosclerosis in the rabbit. Diet modifications and mechanical injury to the artery will be performed. Among the processes being studied are the mechanism whereby diet causes hyperlipidemia in animals, cholesterol absorption by the intestine, and the origin of plasma cholesterol in rabbits subjected to high cholesterol diets.

3. Dietary cholesterol in the development and regression of experimental atherosclerosis

B10 UR 0092162 Merrill, G.F.
Dietary Cholesterol and Coronary Vascular Responses to Histamine

This study is determining whether exogenous histamine can cause reproducible vasospasm in rabbit coronary arteries and whether the vasospasm can be enhanced by high levels of dietary cholesterol. The histamine-induced construction of the coronary arteries is verified by standard ECG tracings and the use of radioactive, tracer microspheres. The effects of dietary cholesterol are being studied.

Z01 HL 04100 Leon, M.
Animal Models of Atherosclerosis

High cholesterol atherogenic diets are fed to swine and rabbits and cardiovascular balloon denudation of the endothelium performed. The object is to establish good animal models for the development of atherosclerosis in order to study the feasibility of in vivo laser angioplasty.

B10 UR 0092162 Merril, G.F.
Responses to Histamine

This study determines vasospastic effect of histamine on rabbit coronary arteries and whether there is an enhancement of this effect by feeding high levels of cholesterol.

R01 HL 25101 Wagner, William D.
Aortic Proteoglycans and Atherosclerosis

The role of aortic proteoglycans in atherosclerosis of the WC-2 pigeon induced by high cholesterol diet is being studied. An attempt is being made to assess genotype environment interaction in the production of proteoglycan aggregates of small mean size or allied composition.

P50 HL 15062 Getz, Godfrey
Specialized Centers of Research-Atherosclerosis (SCOR-A)
Study of Lipoproteins, Artery Wall and Atherosclerosis

As part of the present SCOR a study of atherogenesis in non human primates is being made. Mechanical injury to the vessel wall interacting with dietary induced hyperlipidemia is being investigated.

P01 HL 08974 Strong, Jack P.
Natural & Experimental Atherosclerosis

Experimental studies in non-human primates are being used to study the mechanisms of development and regression of diet-induced arterial lesions. Cholesterol and lipoprotein metabolism is also being investigated in primate animals to obtain an understanding of the mechanisms responsible for the large differences in serum cholesterol and lipoprotein concentrations among animals.

R01 HL 26561 Kaplan, Jay R.
Atherosclerosis: Behavioral/Environmental Influences

The object of this project is to advance understanding of the role of environmental and psychosocial influences and the pediatric precursors of adult atherosclerosis. Cohorts of genetically similar infant and juvenile nonhuman primates are being subjected to a diet moderately rich in cholesterol and salt. Longitudinal measurements including plasma lipid concentrations are made and related to measurements of behavior, physical activity, endocrine factors, growth, etc. Necropsies are being performed to determine content and severity of atherosclerosis.

R01 HL 16587 Malinow, Manuel R.
Synthetic Saponins, Cholesterolemia and Atherosclerosis

The effects of synthetic saponins on atherosclerosis prevention is being studied in rabbits with atherosclerosis induced by a cholesterol diet or a cholesterol-free diet.

R01 HL 24071 Subbiah, Ravi M.
Early Intervention of Age-related Arterial Changes

The objective of this study is a determination of whether dietary intervention started early in life can influence the development of (a) spontaneous atherosclerotic lesions and (b) response to cholesterol-fed atherosclerosis in adult life. The study model is the pigeon. Various aspects of cholesterol metabolism are being studied.

P01 AG 00541 Weksler, Marc E.
Immunological Studies in Aging

The study is designed to investigate the influence of nutrition and exercise on life span, of mice which normally either have a very short or very long life span, and to analyze immulogic changes associated with dietary changes. Mice are fed an atherogenic diet with cholesterol augmentation.

4. Dietary cholesterol and cancer

R01 CA 16750 Broitman, Selwyn A.
Diet, Flora and Colon Tumorigenesis

The stage of action of dietary cholesterol in colon tumorigenesis is to be defined. It is intended to ascertain if the increased cholesterol content of the colonic mucosa resulting from cholesterol feeding disburses cholesterol into the bowel lumen, influences colonic epithelial cell turnover and enhances the permeability of the colon to the "absorption" of a direct acting alkylating agent. The model is the rat.

5. Miscellaneous

B10 UR 007367, Naber, E.C.
Cholesterol Oxidation Products in Foods and Their Effect on Metabolism and Atherogenesis in Chickens

In this study, laying hens are being fed pure cholesterol and oxidized cholesterol (7-keto cholesterol) together with ^{14}C-acetate to measure incorporation in the egg yolk. Both forms increase the incorporation of ^{14}C-acetate into yolk cholesterol, but at the same time increase the total amount of yolk cholesterol.

B10 UR 0069236 Rhee, K.S.
Quality Aspects of Meat Extended with Plant Proteins

One of the objectives of this work is to determine cholesterol content and retention in all meat versus meat-oil seed protein blends.

R01 NS 17994 Morizoro, Tetsuo
High Cholesterol Diet and Auditory Dysfunction

Epidemiological research indicates a correlation between blood cholesterol and sensori-neural hearing loss. This research determines both the direct and indirect effects of a high cholesterol diet on auditory function and structure in an animal model.

C. Division of Research Resources' Multidisciplinary, Resource-related Grants:

M01 RR 00034 Tzagournis, Manuel
Clinical Research Center, Ohio State University

1. Falko, J. - Dietary effects on high density lipoproteins

M01 RR 00036 Kipnis, David
Clinical Research Center, Washington University, St. Louis

1. Gonen, B. - Dietary effects on HDL levels and composition.

2. Schonfeld, G. - Effects of dietary cholesterol on subjects with defects in apolipoprotein E.

M01 RR 00043 Van Der Meulen, Joseph
Clinical Research Center, University of Southern California, Los Angeles

1. Blankenhorn, D. - Cholesterol-lowering atherosclerosis study.

M01 RR 00068 Rowe, Kenneth W., Jr.
Clinical Research Center, University of Cincinnati

1. Jackson, R. - Effect of dietary perturbations on plasma HDL and apolipoproteins.

M01 RR 00071 Kase, Nathan G
Clinical Research Center, Mt. Sinai School of Medicine, NY

1. Ginsberg, Henry - Dietary cholesterol on apoprotein E rich subpopulations of lipoproteins.

M01 RR 00083 Schmid, Rudi
Clinical Research Center, University of California, San Francisco

1. Bersot, T. - Plasma lipoproteins in diet: cholesterol consumption.

M01 RR 00722 Ross, Richard S.
Clinical Research Center, Johns Hopkins University

1. Applebaum-Bowden, D.E. - Dietary cholesterol: effect of estrogens on apolipoprotein E, LDL.

M01 RR 00037 Phillips, Theodore J.
Clinical Research Center, University of Washington, Seattle

1. Bierman, E.L. - Effect of diet on lipoprotein metabolism in diabetic hyperlipidemic subjects.

2. Brown, Gregory B. - Lipoprotein alterations and atherosclerosis progression: diet therapy.

3. Omenn, Gilbert. - Health evaluation of effect of shellfish on cholesterol and lipoproteins in humans.

M01 RR 00102 Kappas, Attallah
Rockefeller University, New York, NY

1. Ahrens, E.H. - Cholesterol metabolism in newborn infant and its mother: breast vs bottle feeding.

2. Brinton, Eliot A. - Drug and diet effects on HDL metabolism.

N01 RR 82118 Castleman, Paul A.
Bolt Beranek and Newman, Inc. Cambridge, MA
Technical Assistance for the Prophet System

1. Garcia, Raymond E. - Regulation of rabbit apolipoprotein metabolism by dietary cholesterol.

P51 RR 00163 Laster, Leonard
Medical Research Foundation of Oregon, Portland, Oregon
Support for Regional Primate Research Center

1. Alexander, Nancy J. - Effects of a high cholesterol diet and insulin on atherosclerosis in rabbits.

2. Malinow, Manuel K. - Steroid balance in cholesterol-fed macaques.

3. Portman, Oscar W. - LDL in arterial wall of squirrel monkeys; diet induced hyperlipoproteinemia.

P41 RR 00862 Field, Frank H.
Physical Chemistry, Rockefeller University, New York, NY
An extended range mass spectrometric research resource

1. Ahrens, Edward H. - 18.O. Isotope Tracer for assay of dietary cholesterol absorption and excretion.

P51 RR 00168 Tosteson, Daniel
New England Regional Primate Research Center, Harvard University

1. Ausman, Lynne M. - Cholesterol turnover and absorption of four monkey species fed semipurified diet.

Identification Codes

Identification codes for the projects listed in this report are shown below.

First three alphanumeric characters indicate the type of award:

B10	Formula Grant
F32	Postdoctoral Fellowship
K01	Special Emphasis Career Development Award
K08	Clinical Investigator Award
M01	General Clinical Research Center
B01	Research Contract
P01	Research Program Project
P30	Center Care Grant
P41	Biotechnology Resource Grant Program
P50	Specialized Center
P51	Primate Research Center
R01	Research Grant
R23	New Investigator Research Award
T32	National Research Service Award Institutional Training Grant
Z01	Intramural Research

Next two characters indicate funding agency:

AG	Department of Health and Human Services, National Institutes of Health, National Institute on Aging
AM	Department of Health and Human Services, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases
CA	Department of Health and Human Services, National Institutes of Health, National Cancer Institute
HL	Department of Health and Human Services, National Institutes of Health, National Heart, Lung, and Blood Institute
HO	Department of Health and Human Services, National Institutes of Health, National Heart, Lung, and Blood Institute
RR	Department of Health and Human Services, National Institutes of Health, Division of Research Resources
UA	United States Department of Agriculture, Agricultural Research Service
UG	United States Department of Agriculture, Cooperative State Research Service
UR	United States Department of Agriculture, Competitive Research Grants Office
VA	Veterans Administration

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